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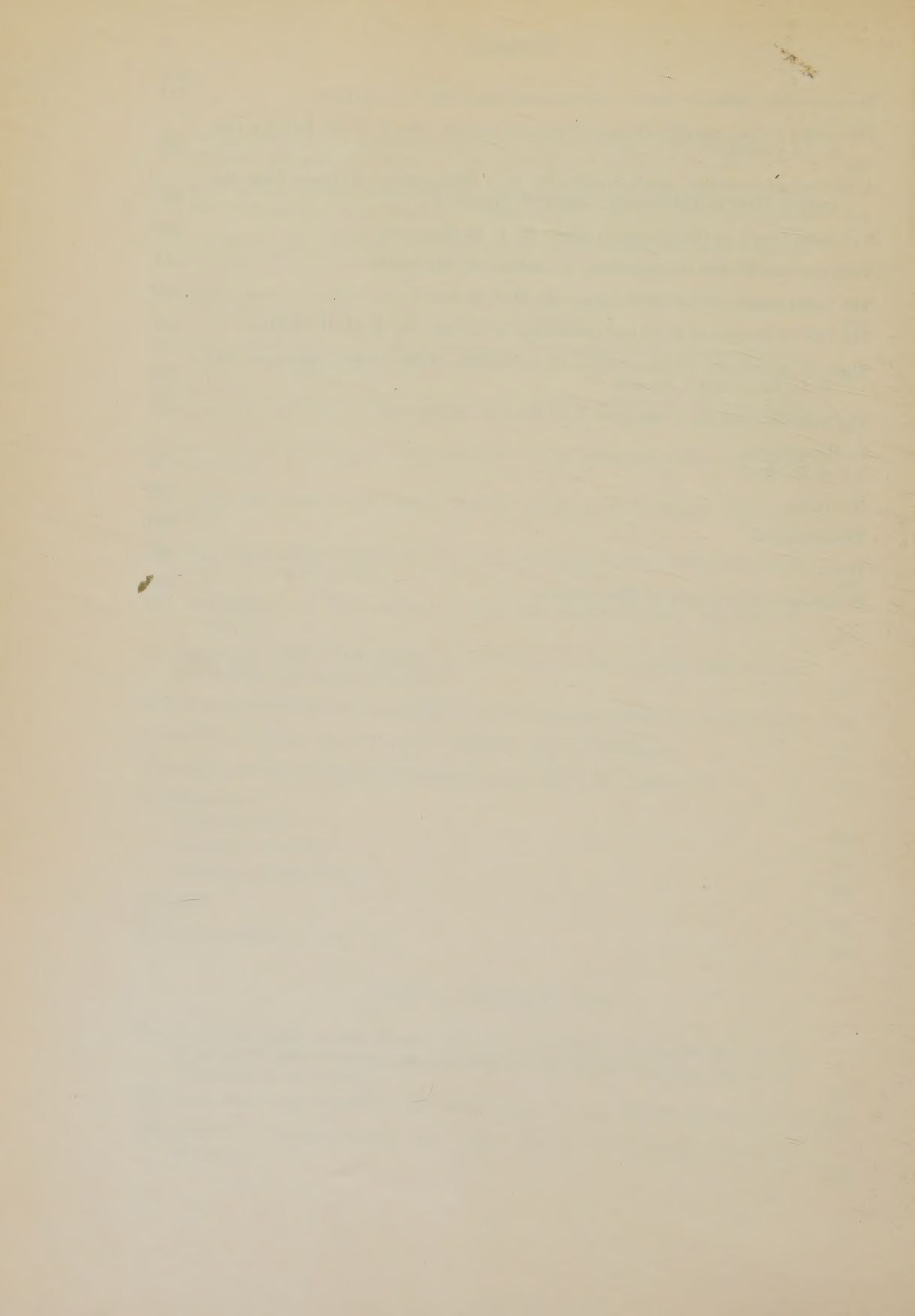
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# THE ANATOMY OF HARE-LIP IN MAN

BY T. SUMMERFIELD KING

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## INTRODUCTION

The anatomical structure of the human upper lip in its early embryonic development is now well known from the investigations of Frazer (1911), Peter (1913, 1949), Hochstetter (1944, 1950), Streeter (1948), Politzer (1952) and others, but there is still divergence of opinion concerning the growth processes involved, for simple embryological investigation has not led to agreement between the various workers. Hence many authors have turned to abnormal human and animal material in an endeavour to clarify the underlying causative factors. Boyd (1932) described the lip in a number of adult mammals, and Veau studied hare-lip in human (1934) and, with le Hyaric (1936), animal preparations and (1942) has successfully bred dogs for the purpose of recovery of embryos at the critical stages of hare-lip formation. Töndury (1950) has personally examined all the known examples of hare-lip in early human embryos. The views of these authors, who give extensive surveys of the relevant literature, will be considered later. Yet, though the morphological structure of the normal and of hare-lip has been described in great detail in the earlier stages, that of the fully developed malformation is less fully known. It is, in fact, only the surface form, osteology and arrangement of the teeth that have been investigated in detail. The failure of macroscopic methods to disclose the anatomy of hare-lip suggested the use of reconstructions from serial sections.

## METHODS AND MATERIALS

The nerves of the face of a full-term foetus with double hare-lip and complete cleft palate, and of a full-term foetus with normal facial development were dissected, using a binocular dissecting microscope. The head of a second full-term foetus with double hare-lip and complete cleft palate, and that of a 7 months still birth with normal facial development were cut horizontally in paraffin at a thickness of  $10\mu$  and every 10th section retained. The normal still-birth sections were all stained with haematoxylin and eosin, and those of the hare-lip specimen were stained alternately with haematoxylin and eosin, and with Van Gieson's iron haematoxylin and picrofuchsin, the latter being more satisfactory for the study of the smaller nerves. From the sections, drawings and graphical reconstructions in coronal and sagittal planes were prepared and courses of the nerves of the face were checked by the dissected specimens.

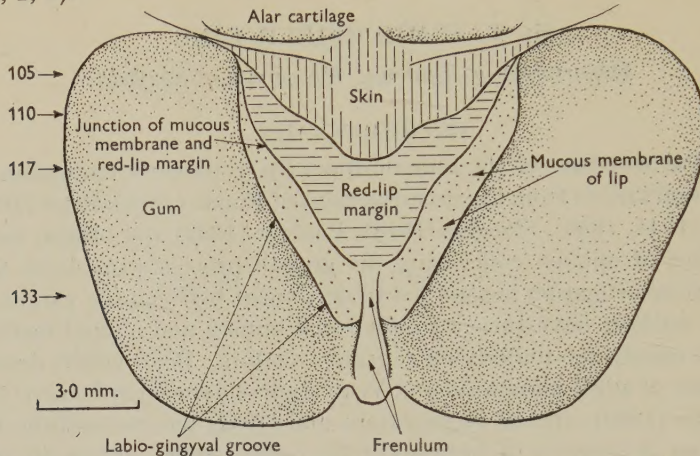
## OBSERVATIONS

### *The surface form and areas*

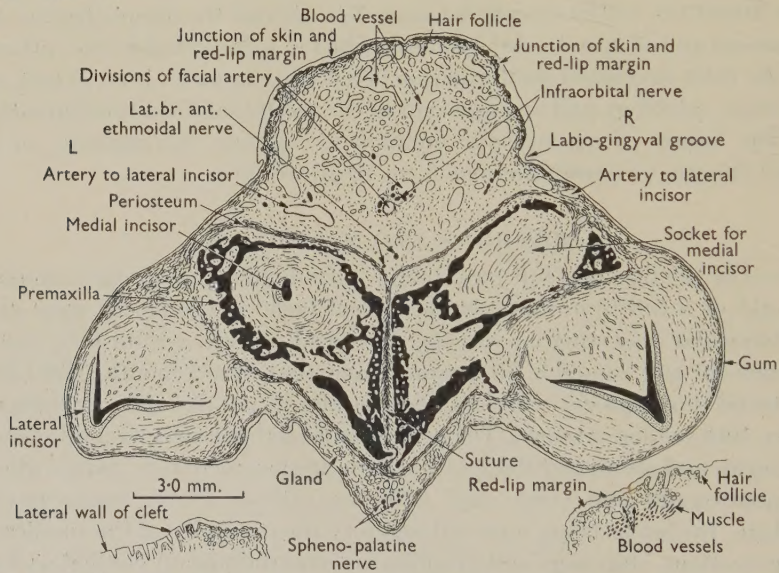
The conditions in the two hare-lip specimens were identical. Two deep clefts continuous with the nostrils separated a median tubercle from the paired lateral elements of the upper lip. To the naked eye the tubercle was dark and shiny and its



covering resembled the mucous membrane lining the mouth. Histological study, however, allowed several discrete areas in the tubercle to be recognized (Text-fig. 1; Pl. 1, figs. 2, 3).



Text-fig. 1. The surface areas of the median tubercle in graphical reconstruction, determined by their histological structure. The numbers refer to sections illustrated in Text-fig. 2, and Pl. 1, figs. 4, 5 and 7.

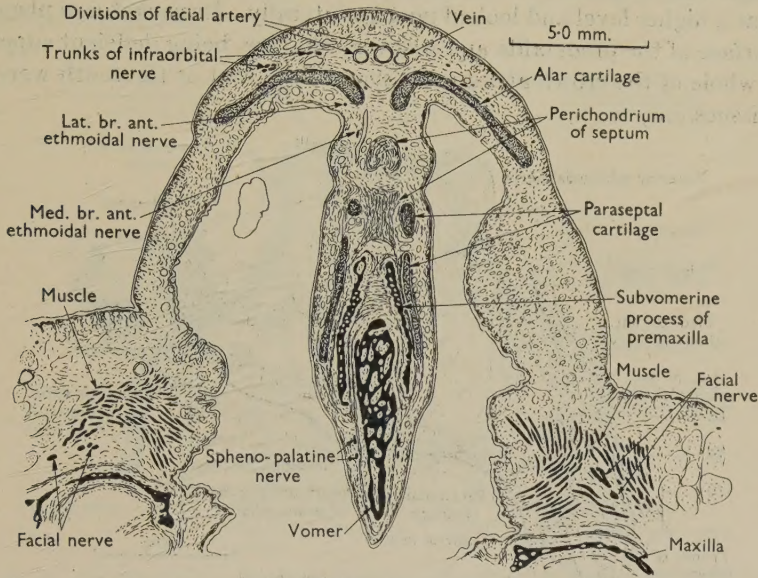


Text-fig. 2. Section 110, cutting the median tubercle at the level of the lateral incisor teeth.

Laterally, the tubercle had two large bulges which, since they contained the incisor teeth and the bone related to them, were clearly parts of the gum (Text-figs. 1, 2). A narrow median ridge crossing the anterior and inferior surfaces of the tubercle between the lateral bulges constituted a frenulum, formed of a connective tissue basis covered by a thick uncornified stratified epithelium (Pl. 1, fig. 7). The frenulum continued into a broad V-shaped area, the red-lip margin. Here the basis was a fibrous connective tissue, very richly vascularized by vessels which could be



distinguished only with difficulty as arteries or veins, though they could be identified by tracing them back to the main trunks (Text-fig. 2). The epithelium had a thin stratum corneum, but there were no glands nor hairs. The region could be identified positively as belonging to the red-lip margin by its large pale epithelial cells in many layers and tall corial papillae (Pl. 1, figs. 4, 5, *r.l.m.* and 6).



Text-fig. 3. Section 98, showing the vomer clamped by the infravomerine processes of the premaxilla, and the large vessels which supply the median tubercle.

Between the red-lip margin and the gum there lay, on either side, a narrower strip of mucous membrane with an uncornified type of stratified epithelium of moderate thickness, identified as everted posterior surface of the lip in spite of the absence of mucous glands (Text-fig. 1; Pl. 1, fig. 5, *m.m.l.*). Superiorly a shallow indentation partly separated this area from the red-lip margin, while inferiorly a deep labio-gingival groove, hidden by the overhang of the area in surface view but visible in the sections, separated this part of the lip from the gum, the epithelium of which was similar but much thinner.

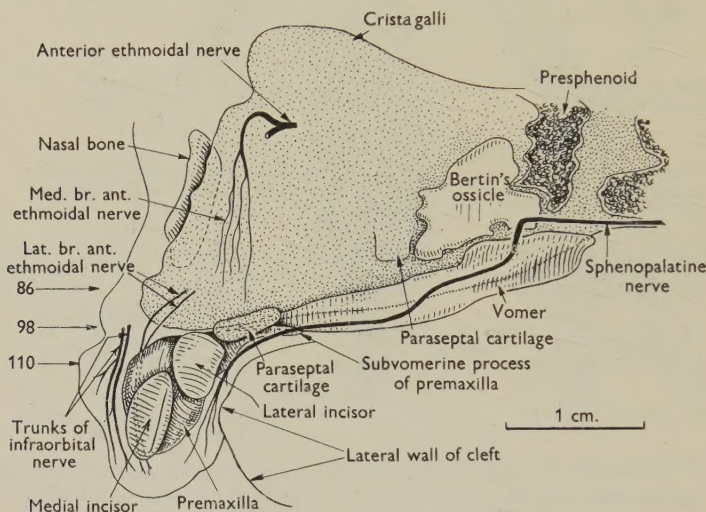
Superior to the red-lip margin was a triangular area covered by hairy skin (Pl. 1, fig. 4, *sk.*). The part of this area between the nostrils formed a wide columna, an identification confirmed by its close relation to the inferior margin of the cartilaginous nasal septum. The more inferior part, remote from the septum, presumably represented the skin of the median philtral segment of the lip, though in this specimen no boundary could be found between these parts. Near the red-lip margin, though the epithelium was still clearly of the ordinary skin type and could still be distinguished with certainty from the red-lip itself, hairs were scanty or absent (Text-fig. 2). The areas on the photographs of the median tubercle (Pl. 1, figs. 2, 3) have been labelled from the histological findings.

The tubercle was attached superiorly to the nasal septum (Text-fig. 6). The only mucous glands found in the tubercle were on its posterior surface.



*The Bones*

The median tubercle contained the right and left premaxillae united by a median suture. Each premaxilla was enlarged laterally to carry the two incisor teeth. The central incisor looked infero-laterally, and its socket, though shallow compared with the normal, was complete around the base of the tooth. The lateral incisor was situated at a higher level and looked postero-laterally. Its socket was placed on the lateral surface of the premaxilla and was very shallow, being deficient superiorly, so that the whole of the crown and a great part of the root of the tooth were covered by soft tissues only.



Text-fig. 4. Graphical reconstruction of the structures near the mid-plane in lateral view. The numbers refer to the sections shown in Text-figs. 5-7.

Near the mid-line the premaxilla was raised to form a narrow paramedian ridge, the subvomerine process, which with its fellow formed a groove for the lower border of the cartilaginous nasal septum, and was prolonged backwards on either side of the vomer (Text-figs. 3, 4).

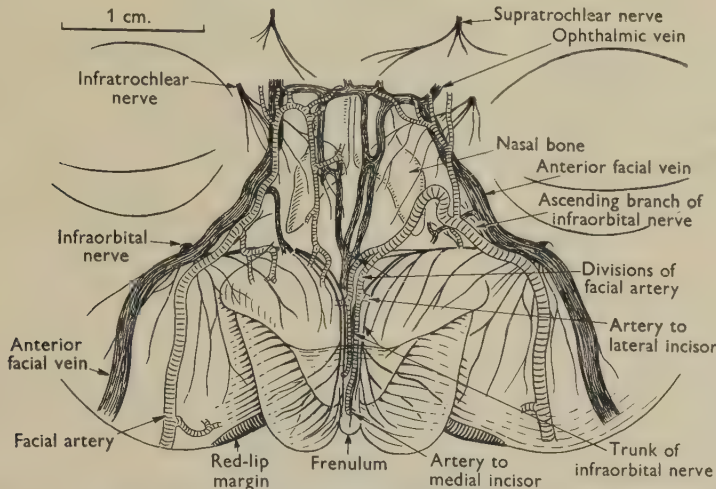
The maxilla was represented on each side by a shallow alveolar process containing some of the milk teeth, a canine and two molars, and by a frontal process and a pair of very minute palatal processes. There was no maxillo-premaxillary (incisive) suture, for the bones were widely separated throughout.

The enamel organ for the permanent medial incisor was placed postero-medial to the deciduous tooth, and was embedded in bone. It appeared normal in size. That for the lateral incisor was smaller and was placed directly deep to the shallow socket of the deciduous tooth. The enamel organs for the other permanent teeth appeared normal.

The vomer was long and narrow, with a short lower border inserted between the subvomerine processes of the premaxilla (Text-figs. 3, 4). The lower border of the vomer sloped from the presphenoid to the premaxilla. The vomer was formed for the most part by two lateral wings, united posteriorly, and the cartilaginous nasal septum was enclosed at its postero-inferior border by these wings.

*The nerves*

On each side the long sphenopalatine nerve passed down on the side of the vomer, lying near its postero-inferior border, to reach the posterior part of the median tubercle. It was distributed to the mucous membrane over the vomer and the posterior and postero-lateral parts of the premaxilla. It was not distributed to the incisor teeth (Text-fig. 4).

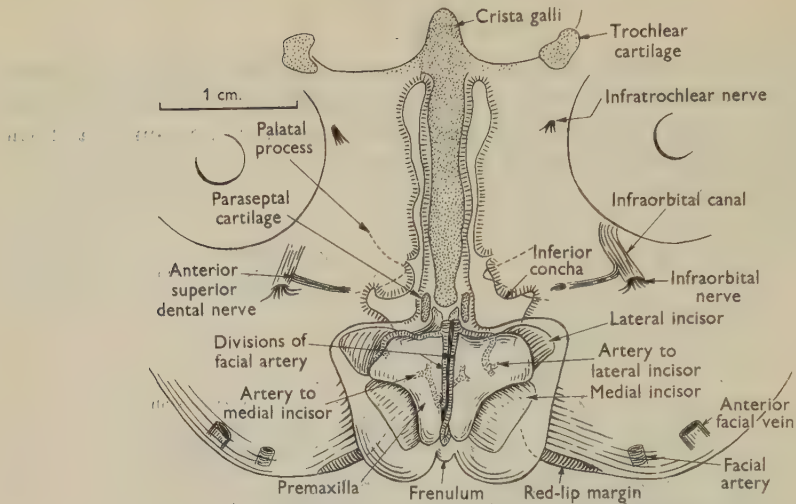


Text-fig. 5. Graphical reconstruction of the superficial nerves and vessels of the face in anterior view.

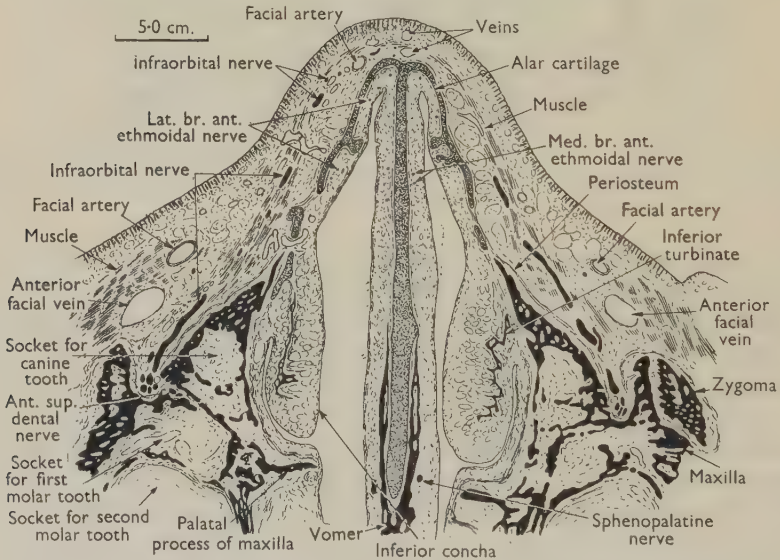
The anterior ethmoidal nerve passed almost vertically downwards in two divisions, a medial and a lateral (Text-figs. 3, 4 and 7). The medial division ran on the anterior part of the septal cartilage, dividing into several branches, and its terminal twigs reached the lower part of the septum where they ended, none being traceable into the upper lip. From the lateral division, branches were given off at fairly regular intervals and passed downwards and forwards to supply the lateral wall of the fossa anterior to the nasal conchae. A few minute twigs were traced into the upper part of the median tubercle, just anterior to the attachment of the septal cartilage to the premaxilla, where they supplied the ridge identified as the frenulum labii. As this was unexpected, the corresponding nerves were traced out in the normal material, and there again the lateral branch was followed to what was undoubtedly the frenulum, so confirming the identification in the hare-lip specimen. The external nasal branch of the anterior ethmoidal was looked for carefully, but though it was present in the normal material both in the microscopic and macroscopic preparations it could not be found in the abnormal specimen, its place being taken by the greatly enlarged infraorbital trunks.

The infraorbital nerves, besides spraying out to supply the cheeks, gave off on each side two large trunks which passed horizontally and medially across the face to the side of the nose (Text-fig. 7), and then arched inferiorly (Text-fig. 5) to reach the columna naris where they formed neuro-vascular bundles with the divisions of the





Text-fig. 6. Graphical reconstruction of the deeper structures in anterior view.



Text-fig. 7. Section 86, showing the infraorbital nerves passing across the face.

facial artery (Text-fig. 2). They traversed the columno-philtral region to end in the main mass of the median tubercle where they supplied the mucous membrane but not the teeth.

No similar nerves were found in the dissected normal material, but in the sectioned normal specimen a few filaments of the infraorbital nerve could be traced around the nostril, traversing the ala and columna naris, to reach the philtrum, though these nerves were very small.



The upper part of the nose and interorbital regions were supplied by the supra-trochlear and infratrochlear nerves; both of which were well developed (Text-fig. 5).

The incisor teeth received no nerve supply. The posterior dental nerve was traced to supply the molars and the canine, where it ended. It was considered a possibility that the incisor teeth might receive a supply from the cutaneous nerves, or from those passing to the mucous membrane of the gum (the infraorbital and the sphenopalatine), but this was found not to be the case. The anterior superior dental nerve was found in a canal in the maxilla, leading from its origin from the infraorbital nerve (Text-figs. 6, 7). It passed medially in the bone and then emerged on the cheek and ended by joining one of the infraorbital trunks. The greater palatine nerve had an abortive course, passing forwards on the medial side of the maxilla towards the diminutive palate and ending blindly about half-way along the maxilla.

In the dissected hare-lip specimen (Pl. 1, fig. 8), the median tubercle again received its nerve supply by branches from the infraorbital nerves which passed over the side of the nose and down the diminutive columna naris, crossing superficial to the lower part of the small external nasal nerve.

#### *The blood vessels*

The arterial supply was derived from the facial arteries (Text-fig. 5). The right facial artery gave a descending branch to the ala and another branch to the dorsum of the nose but took no part in the supply of the median tubercle. The left artery formed a horizontal arch which crossed the lower part of the nasal bone and then divided as it reached the mid-line into two divisions which passed down the dorsum of the nose, side-by-side, to the philtral region. Here each division gave a branch which wound over the body of the premaxilla to reach the shallow alveolus of the lateral incisor and enter the pulp, in which it formed a rich arborescence. The two divisions then passed along the attachment of the frenulum and ended, the right by dividing to supply both medial incisor teeth (Text-fig. 6), the left, after turning posteriorly below the premaxillae, by anastomosing with the sphenopalatine arteries which accompanied the sphenopalatine nerves.

The veins accompanied the arterial trunks, but were somewhat plexiform in arrangement, intercommunicated with each other, and ended in the facial veins of both sides by arches which were placed considerably higher than the single arterial arch. They also made large communications with the ophthalmic veins passing below the trochlear cartilages (Text-fig. 5).

#### *The muscles*

Muscle fibres were well developed over the cheeks and approached the red-lip margin on the lateral face of the cleft. They covered the upper part of the nose where a few fibres crossed the mid-line (Text-fig. 7), but they were absent in the ala near the nostril (Text-fig. 3), and in the columna naris. No muscle fibres were found in the median tubercle (Text-fig. 2). The branches of the facial nerve followed the distribution of the muscles, but did not spread so far.

## DISCUSSION

*The surface areas*

The specimen of hare-lip described here in detail seems typical of its kind for the various surface features, including the lateral bulges, the short thick columna naris and the conspicuous frenulum are seen in Peter's (1913) fig. 179 and Veau's (1934) fig. 5, and Boyd (1932) has commented on the presence of a frenulum in such cases. In some specimens the lip is more distinct, as in Veau's (1934) fig. 3, and in the specimen figures by Hamilton, Boyd & Mossman (1952, fig. 76B), and in others the whole median tubercle is reduced to a small projection, as in Veau's (1926) fig. 7. The relatively small size of the lip as compared with the gum is possibly due to a lack of expansion by ingrowing muscle, for, as is usual in such cases (cp. Veau, 1926, fig. 8), no muscle fibres were found in the median tubercle, though they were plentiful in the cheek and over the side of the nose. Whether muscle fibres are in fact found in these cases of double hare-lip that have well-developed philtral regions could not be determined for lack of suitable material. It might be possible, however, for muscle to grow into the lip via the columna naris, for in cases of unilateral hare-lip the muscle fibres of the sound side grow across the mid-line so as to invade the tissues of the other side (Veau, 1926).

The presence of a hairy median area does not support Boyd's (1932) view that the medial nasal processes become buried beneath the maxillary processes in a manner similar to that seen in rabbits, so that the human upper lip is formed exclusively of maxillary process tissue, for it would appear to be most unlikely that an area of potentially hairy skin should be overgrown by a superficial layer of maxillary tissue. The present specimen does, however, confirm the view of Veau & le Hyaric (1936) as to the difference between the human lip and that of animals. In the latter, such as the cat and dog, the maxillary processes reach the mid-line but may not fuse with each other, and the parts derived from the medial nasal processes are devoid of hairs. In man the maxillary extensions do not overgrow the medial nasal eminences and the latter become hairy giving the condition characteristic of haplorhine primates as opposed to the strepsirhine condition characteristic of lemurs and non-primate mammals (Jones, 1948). In the hare-lip material, the medial nasal processes are isolated, but still develop hairy skin.

The absence of mucous glands in the median tubercle anterior to the line of the teeth is puzzling, though confirmed by Veau's (1926, fig. 8) section from a similar specimen which also had a conspicuous frenulum but no muscle. The absence might be taken as favouring his suggestion of an origin from the maxillary process of the tissues of the oral surface of the lip where these glands usually occur, but it might be possible that the glands are not developed because the lip itself is dwarfed, or possibly because no parasympathetic nerves have reached this part of the tubercle.

*The cutaneous nerves*

The distribution of the cutaneous nerves in the adult has often been used as a guide to embryonic processes of development (Frazer, 1940). The ophthalmic nerve by its nasociliary branch (eventually the anterior ethmoidal nerve) was regarded as the nerve of the frontonasal process, and the infraorbital as the nerve

of the maxillary process. Thus Frazer maintained that maxillary mesoderm passed upwards across the face to the side of the nose, while another portion of this mesoderm passed medially below the nose to form the upper lip, so that the infraorbital nerve came to supply these areas. The long sphenopalatine nerve was associated with maxillary mesoderm which passed inwards on to the roof of the nasal fossa, and then spread downwards over the nasal septum where it formed the vomer, carrying the nerve with it.

It would indeed be convenient if the movements of the mesoderm in the embryo could be inferred from the distribution of the sensory nerves in the adult, but though in the early embryo some of the mesodermal proliferations appear to be built around particular nerve endings, there is no reason to believe that nerves cannot extend beyond their particular fields in later development. There is no direct embryological evidence in favour of Frazer's supposed wanderings of the maxillary mesoderm, and Töndury (1950) has maintained that the facial processes possess neither their own vessels nor nerves.

The present specimens show that under abnormal conditions the sensory nerves can wander far from their normal courses. In both the examples studied the infraorbital nerves gained the ridge of the nose near the mid-line, an area usually supplied by the anterior ethmoidal nerve, and so passed through the columna naris to the philtral region. The nerves could have paid no regard to the boundaries of the early embryonic processes, for it seems unlikely that the tissues of the median tubercle reached it by following such a peculiar course. The finding in the normal material, however, of a few small branches of the infraorbital nerve passing from the ala to the columna naris suggests that the peculiar trunks in the hare-lip specimen are formed by the enlargement of a normal nervous pathway, rather than being entirely new structures. Recently Politzer (1952) has reviewed the development of the face, both normal and abnormal, and shown that no valid conclusions can be drawn regarding the development from the study of, for example, oblique facial fissures, which are of the nature of irregular tears rather than failures of union. He suggests that attempts in text-books to define embryonic areas on the adult face should be discontinued.

#### *The blood vessels*

The arterial supply of the median tubercle has long been doubtful. Veau & Burgeat (1926) found, from the study of three fetuses cut in series and several dissections, that in double hare-lip the sphenopalatine arteries were not hypertrophied and that in unilateral cases these arteries were of equal size, so that the supply could not be derived from them. Yet Veau (1926) found that the tissues of the median tubercle bled copiously when cut, and that the bleeding was arterial in type. Both Veau (1926) and Cadenat (1931) failed to find how the blood reached the tubercle, and the point does not seem to have been considered by later authors.

Whether the arching vessel described here always occurs on one or both sides in bilateral hare-lip cannot be stated without further study. It would be difficult to recognize in dissections of uninjected material, and even in the sections it had to be followed for some distance before it could be distinguished from the veins, so that it is not suprising it has not been found except in the long series of large sections used in the present study.



Again it is not known whether the arching artery is a normal embryonic structure which persists in cases of hare-lip, or whether it is an altogether new formation. Preliminary investigation of normal foetuses suggests the former interpretation.

Both sphenopalatine arteries anastomosed with one of the terminal divisions of the facial arch, but were of very small size. As there was no incisive canal to traverse, the arteries passed with the nerves directly to the posterior surface of the median tubercle. They were placed symmetrically, not one anterior to the other as was long believed, to the usual position, though Bellairs (1951) found them always symmetrically placed in the normal.

#### *The premaxilla*

In animals with hare-lips the premaxilla is usually ossified in two parts, one medial resembling the human form of premaxilla, and the other a small bone assisting in the formation of the lateral margin of the nasal aperture (Veau & le Hyaric, 1936). This lateral bone was sought in the sectioned specimen, but was not found. Occasionally a small bone is found in this position or wedged between the nasal bone and frontal process of the maxilla in normal and in hare-lip material, but this may be a naso-maxillary bone such as is normally found in several primates, rather than a part of the premaxilla (Ashley-Montagu, 1935).

The subvomerine processes, which have independent centres of ossification, appear normal, though since the naso-palatine canals are not formed owing to the failure of fusion leading to hare-lip formation, the relations of the processes are altered. In the normal foetus the two processes lie medial to the divided naso-palatine ducts (Vallois & Cadenat, 1926), while in hare-lip the canals are not formed and the processes lie directly beneath the mucous membrane of the clefts.

Woo (1949) and others have found that in the normal foetus of about 21 mm. the premaxilla ossifies as a separate centre related to the incisor teeth, but soon joins the maxillary centre. In hare-lip the corresponding centre is seen to form the alveolar region with complete or incomplete sockets, and to join the subvomerine centres posteriorly, but to form no palatal or facial processes of its own. The question as to whether the absence of such processes is due to an inability of the bone to spread across a cleft, or to the non-participation of the premaxilla in their formation, cannot be decided from hare-lip material, but Jarmer's (1922) demonstration that the premaxillary ossification normally invades maxillary tissues would support the former interpretation. The abnormal width of the nasal aperture in hare-lip may, as Veau (1926) suggested, be due to the absence of premaxillary facial processes.

#### *The blood and nerve supply of the incisor teeth*

Veau (1926, 1934), and others, have noticed that in bilateral hare-lip the incisors, especially the lateral incisors, are usually lost in the early years of life, and have ascribed this to a deficiency in blood and nerve supply. In the present specimen the blood supply, derived from the arching trunk of the facial artery, is abundant, but there is no nerve supply, and the sockets are poorly formed, particularly that of the lateral incisor. These two last factors are presumably responsible for the insecurity of the teeth.

The anterior superior dental nerves are represented by well-developed bundles.

Unable to reach the incisors by the usual routes, for these are involved in the cleft formations, they join the trunks of the infraorbital nerves, and so presumably reach the incisor region. Why, having reached it, they do not in fact supply the teeth is quite obscure.

#### SUMMARY

1. The anatomical structure in bilateral hare-lip with cleft palate is described from sectioned and dissected material.
2. The premaxillae had shallow sockets for the incisor teeth. Subvomerine processes were present, but no palatal or facial processes.
3. The arterial supply of the median tubercle was derived from the left facial artery by an arching trunk which crossed the nasal bone to reach the mid-line and then traversed the columna naris and philtrum. The veins had similar arches, but emptied into the anterior facial veins on both sides.
4. Large trunks of the infraorbital nerves passed down with the arterial trunks to the median tubercle.
5. The incisor teeth had a copious blood supply from the facial arch, but no nerve supply. The anterior superior dental nerves of each side, after traversing the maxillae, joined the infraorbital trunks.
6. The normal development of the philtral portion of the upper lip and of the premaxillary region is discussed in the light of the findings in hare-lip material.
7. In abnormal conditions nerves may wander far from their normal courses, and the distribution of sensory nerves cannot be used safely for the determination of embryonic sources of adult tissues.

I wish to thank Prof. Francis Davies and Dr R. Wheeler Haines for their help and advice, and also Mr J. H. Kugler, who prepared the sections and photographs.

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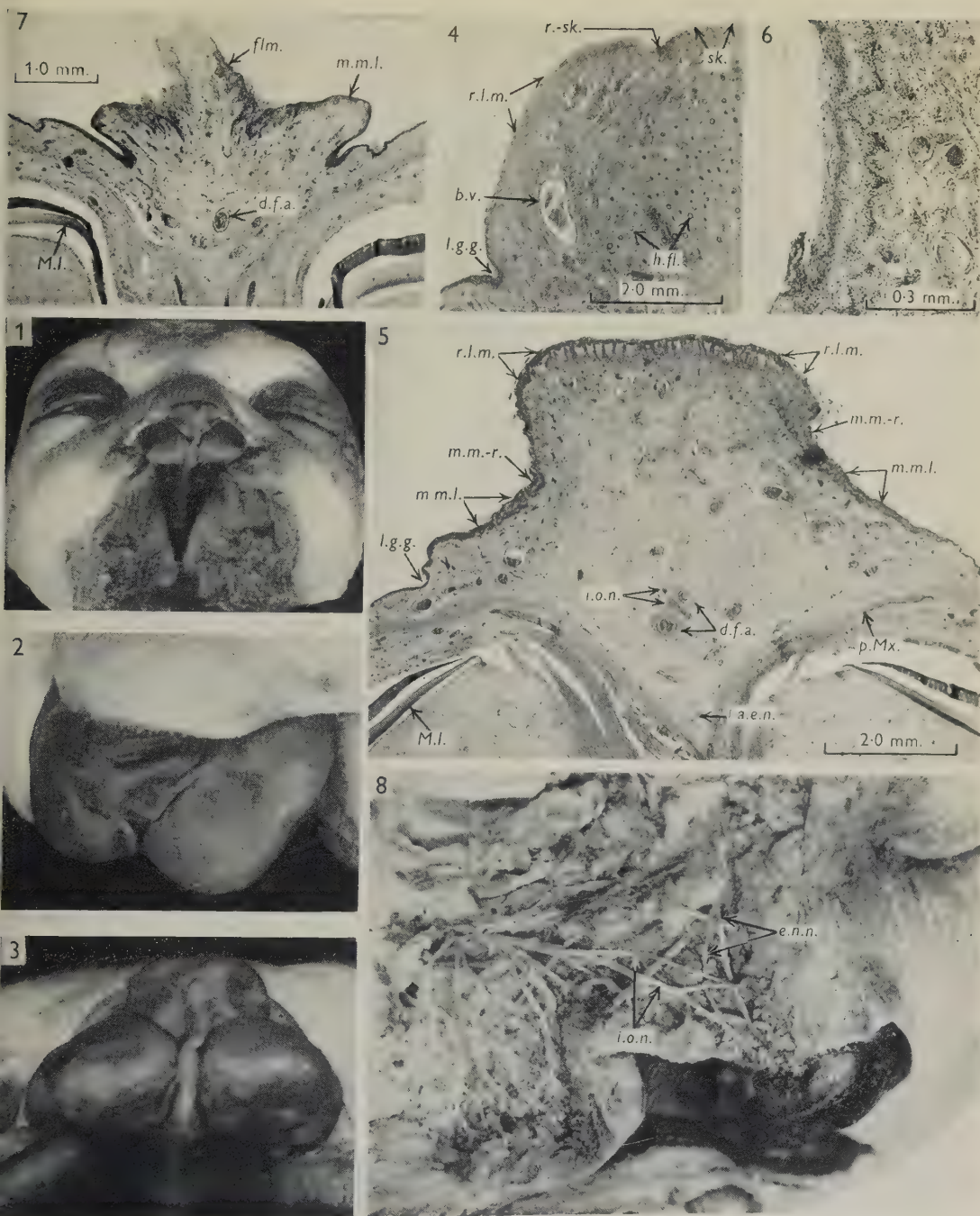
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## EXPLANATION OF PLATE 1

- Fig. 1. Inferior view of block.
- Fig. 2. Oblique view of median tubercle.
- Fig. 3. Inferior view of median tubercle.
- Fig. 4. Section 105, through upper part of median tubercle, showing hair follicles, red-lip margin, mucous membrane and labio-gingival groove. No muscles present.
- Fig. 5. Section 117, through median tubercle below the hairy region, showing red-lip margin and everted mucous membrane of lip.
- Fig. 6. Enlargement of red-lip margin to show structure of epithelium.
- Fig. 7. Section 133, through lower part of median tubercle, showing frenulum and partly everted mucous membrane of lip.
- Fig. 8. Dissection of hare-lip specimen showing the infraorbital nerve passing round the ala to reach the median tubercle, crossing superficial to the external nasal nerve.

## EXPLANATION OF LETTERING

<i>b.v.</i>	blood vessel	<i>M.I.</i>	medial incisor
<i>d.f.a.</i>	division of facial arch	<i>m.m.l.</i>	mucous membrane of lip
<i>e.n.n.</i>	external nasal nerve	<i>m.m.-r.</i>	junction of mucous membrane and red-lip margin
<i>flm.</i>	frenulum	<i>p.Mx.</i>	premaxilla
<i>h.fl.</i>	hair follicle	<i>r.l.m.</i>	red-lip margin
<i>i.o.n.</i>	infraorbital nerve	<i>r.-sk.</i>	junction of skin and red-lip margin
<i>l.a.e.n.</i>	lateral branch of anterior ethmoidal nerve	<i>sk.</i>	skin
<i>l.g.g.</i>	labio-gingival groove		





## HAIR REPLACEMENT IN THE DOMESTIC RABBIT

By H. J. WHITELEY\* AND F. N. GHADIALLY

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It is now well known that hair replacement in the smaller rodents is discontinuous, occurring in cyclical waves of active growth spreading from the venter to the dorsum. This was first observed by Dry (1925-6) and confirmed by Wolbach (1951) in the mouse, and a similar cycle was demonstrated in the rat by Haddow, Elson, Roe, Rudall & Timmis (1945) and Durward & Rudall (1949). There is little information, however, concerning hair replacement in the domestic rabbit, but Hale (1945), after observing the pattern of areas of hair growth on the trunk of the shaved rabbit, came to the conclusion that the growth cycle started on the dorsum and progressed downwards to the venter. A similar cycle was observed in the spring moult of the varying hare by Lyman (1942), the autumn moult, however, spreading from the venter to the dorsum. Our observations (Whiteley & Ghadially, 1951) were in agreement with those of Hale (1945), and it was decided to investigate more fully the mode and pattern of hair replacement on the trunk, not only in the shaved animal, as the stimulus of shaving might affect the normal pattern, but also in the intact animal. This problem is of interest because of its intrinsic biological significance, and also as accurate information of normal hair replacement is essential for the proper study of substances affecting hair growth. Further, there may be a possible relationship between the distribution of skin lesions and the metabolic activity of the skin, which in the rabbit does not appear to be uniform (Whiteley, Stoner & Threlfall, 1953).

## METHODS

Ten adult and five young albino rabbits were dyed with Inecto Rapid No. 1 deep black (except for the face). The technique consisted of diluting the dye with equal amounts of 10 vol. %  $H_2O_2$  and gently rubbing into the fur. After the solution was applied the animals were placed in front of an electric fire and the hair was repeatedly combed during drying. This was found necessary in order to prevent matting of the hair. After being treated in this manner the hair was a brownish black colour right to the roots. The skin did not appear to be affected and the fur retained its natural texture. The animals were kept under observation in individual cages and were fed the usual mixed diet. The process of hair replacement was recorded photographically on 35 mm. film at monthly intervals. When there was complete replacement the animals were redyed and the following cycle studied in a similar manner. The experiment lasted for 18 months.

A group of four adult Agouti rabbits were repeatedly clipped, removing the hair encircling the trunk. After approximately 5 months all hair growth ceased and the skin became inactive and pink, and the animals were observed for a further 9 months.

Besides these animals, numerous agouti and chinchilla rabbits, used for other

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experiments, have been examined by blowing the fur apart on the trunk, when the dark zones of hair growth are easily observed. Subsequent clipping has revealed characteristic patterns.

## RESULTS

### *A. In the dyed rabbit*

It was found in the adult albino rabbit that the hair replacement on the back and flanks occurred in a progressive cycle, while the hair replacement over the abdomen occurred independently of this dorsal cycle. In five of the animals the dorsal and lateral hair was replaced annually, starting in the autumn, while in three others annual hair replacement started in the spring. In the remaining two the cycle was bi-annual, occurring in the spring and autumn. The duration of these cycles varied from 2 to 7 months, but most of the animals took an average period of 3–4 months to complete the cycle. Between these cycles of growth there was a period of complete quiescence of about 8–9 months.

The dorso-lateral hair replacement cycle followed a characteristic pattern, seen only in its proper sequence in those animals that were dyed in the quiescent phase. Here the hair replacement cycle occurred in the dorsal region, starting usually near the neck (Pl. 1, figs. 1, 6) or as irregular patchy growth along the dorsal surface of the rabbit (Pl. 2, fig. 11).

Within a matter of weeks the wave of hair growth had spread over the major part of the dorsum and was beginning to progress laterally over the flanks (Pl. 1, figs. 2, 3, 7; Pl. 2, fig. 12). This downward progression continued to the ventro-lateral aspect (Pl. 1, figs. 4, 8; Pl. 2, fig. 13) and subsequent cycles showed a similar pattern of replacement (Pl. 1, fig. 5). It will be noticed that there is complete replacement of the originally dyed hair by the new growth of hair.

If, however, the animal was dyed, not in the quiescent phase, but when a wave was in progression, the wave of replacement will be observed in a different position (Pl. 2, fig. 9). However, its progression downwards is still observed (Pl. 2, fig. 10).

In the young animal (3 months) the appearance differed from that of the adult in that the characteristic dorsal initiation and lateral progression was lacking; the hair appeared to grow diffusely over the trunk flecking the dyed areas with white (Pl. 2, figs. 14, 15), while in the following year the hair replacement followed the adult pattern (Pl. 2, fig. 16).

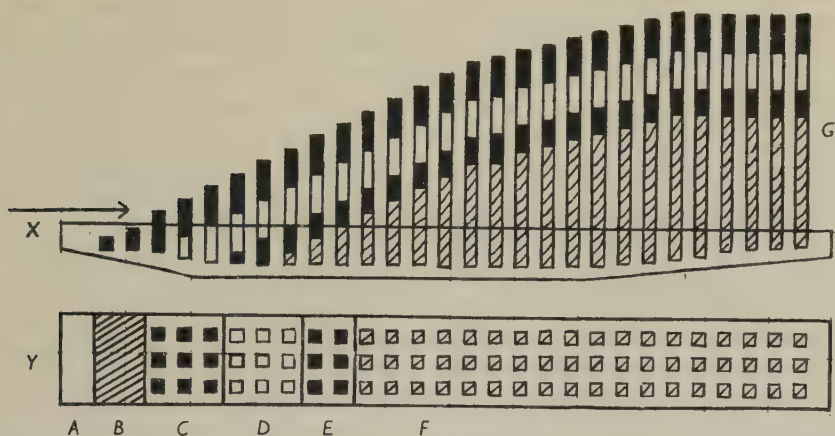
The hair on the ventral surface was replaced annually during the winter in seven adult rabbits, and in three there was a bi-annual replacement in the winter and summer. The duration of the replacement was 4–6 months (Pl. 3, figs. 17–20). Although replacement of hair on the limbs was not studied in detail, it appeared, in some animals, to occur independently of the dorsal and lateral cycles (Pl. 1, figs. 7, 8; Pl. 2, figs. 9, 10).

### *B. Observations in the clipped animal*

Of the four clipped animals observed, one showed a mid-dorsal growing zone (Pl. 3, fig. 21), two showed laterally symmetrical growth zones (Pl. 3, fig. 22), and the remaining animal showed no growth zones but developed a mid-dorsal growing zone within a fortnight. Examination of the lateral band of hair growth in the chinchilla and agouti rabbit revealed a characteristic pattern (Pl. 3, fig. 23) which



could only adequately be explained by assuming that the hair replacement wave progresses downwards from the dorsum, the hair having been cut through at different levels as it penetrates through the skin (Text-fig. 1). After a varying period of time it was noticed that a wide zone of pink skin, immediately ventral to the growing edge, became uniformly pigmented and active hair growth followed. However, no hair growth occurred in the region immediately dorsal to the lateral growing zone during this time. After these observations had been made the animals were repeatedly clipped to remove any regenerating hair, and eventually after a period of 5 months all hair growth ceased and the skin became uniformly pink.



Text-fig. 1. Diagram of the lateral band of hair growth to explain the appearance seen in Pl. 3, fig. 23, and illustrate the ventral progression of the dorso-lateral wave of hair replacement. *X*=section through the lateral band of hair growth; *Y*=surface view of the band, the hair having been removed at level→; *A*=pink ventral skin; *B*=pigmented skin, no hair protruding; *C*=pigmented skin, black shafts protruding; *D*=non-pigmented skin, white shafts protruding; *E*=pigmented skin, shafts protruding; *F*=broad pigmented band tailing off to grey, shafts protruding, and then pink quiescent dorsal skin; *G*=outer-coat hair of chinchilla fully grown (black tip white band, black band tailing off to grey).

The animals remained bald for about 7 months even though they were, during one part of the experiment, placed outdoors during the winter for 8 hr. daily for a fortnight. In the early spring mid-dorsal areas of hair growth developed which progressed down over the flank. These findings have been amply confirmed by observations on many animals that have been clipped for other experimental purposes.

#### DISCUSSION

The results confirm and amplify the original observations of Hale (1945). It would appear that the method of hair replacement in the rabbit is essentially similar in principle to that in the mouse and rat, in that it is cyclical. There are, however, minor differences, the wave of replacement starts in the dorsum, and there is independent replacement of the hair of the belly. Butcher (1951) states that in the rat the new hair does not push the old hair out, but grows alongside it, while this is seen in the young rabbit, it does not occur in the adult, where the replacement is complete.

In an investigation into the underlying mechanism of this cyclical growth in the rat, involving partial X-ray depilation and the rotation of rectangles of skin of the flank through 180°, Durward & Rudall (1949) came to the conclusion that this growth phenomenon represented a fixed genetic pattern, and that follicles passed through an active phase, associated with an increased blood supply also seen in the rabbit (Whiteley *et al.* 1953) and a quiescent phase. Further, during the resting phase it was found that no stimulus producing an increased blood supply would initiate hair growth until the resting or refractory state had been completed. In this connexion our observations in the rabbit are of interest. The dark zone of early follicular activity preceding the wave front is narrow, as compared with the wide zone appearing a few days after removal of the hair, suggesting that the stimulus of hair removal triggers off a larger zone of growth in the potentially active skin preceding the wave front. The skin behind the growth wave is functionally very different from this potentially active skin, for while removal of hair produces growth in the latter it does not in the former. Wounding of the skin on both sides of the growth wave further demonstrated this profound difference for while there was prolific hair growth in the pre-active zone there was little evidence of activity in the post-active zone. These findings support Durward & Rudall's hypothesis that the hair follicle passes through active and resting phases, the resting phase being at first completely refractory and later potentially active. While inherent genetic factors are no doubt very important, it would appear that these can be modified to some extent by the internal environment, for in the rat thyroidectomy will occasionally cause reversal of the growth wave (Dieke, 1948), and our results in the rabbit suggest a seasonal variation.

Further study of this cyclical activity is in progress, as it is thought that the reaction of these zones to varying pathogenic agents might provide valuable information concerning the distribution and localization of skin lesions.

#### SUMMARY

Observations on a series of adult and young dyed albino animals and clipped agouti and chinchilla rabbits revealed a characteristic pattern of hair replacement. This replacement occurs in cycles which may be annual or bi-annual. There is a dorso-lateral wave of replacement starting on the dorsum and spreading laterally over the flanks, and in the pigmented animal there is a characteristic wave margin and pattern to the lateral band of replacement. Further, there is an independent cycle of hair replacement on the belly. In the young rabbit the replacement is not complete, as it is in the adult, and the areas of active growth are more diffuse.

We wish to express our thanks to Prof. H. N. Green for advice and criticism and to Messrs Rapidol Ltd., for the supply of Inecto Rapid (No. 1) hair dye.

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# EXPLANATION OF PLATES

In all figures in Pls. 1 and 2 and Pl. 3, figs. 17-20 white areas, excluding the face, are areas of new hair growth.

## PLATE 1

- Fig. 1. Adult albino rabbit (no. 36). Dorsal view showing the commencement of the hair replacement cycle, starting behind the neck and from a few mid dorsal foci after an initial quiescent period of 4 months after the application of the dye.
- Fig. 2. Rabbit (no. 36). Dorsal view 5 weeks later showing progression of the replacement cycle over the back and on to the flanks.
- Fig. 3. Rabbit (no. 36). Lateral view (same time as fig. 2) showing extension of the hair replacement on to the flanks.
- Fig. 4. Rabbit (no. 36). Lateral view after a further period of 4 weeks. The cycle has extended further down the flank and over the rump. Note that the hair replacement is complete. The dorsal cycle was completed after a further 5 weeks.
- Fig. 5. Rabbit (no. 36). Lateral view the following year showing a similar pattern of replacement to that seen in fig. 3 during the course of the subsequent hair replacement cycle.
- Fig. 6. Adult albino rabbit (no. 70). Dorsal view showing commencement of the autumn replacement cycle (behind the neck) after an initial quiescent period of 2½ months.
- Fig. 7. Rabbit (no. 70). Lateral view showing progression of the hair replacement cycle after a further period of 3 weeks.
- Fig. 8. Rabbit (no. 70). Lateral view after a further period of 6 weeks. There has been progression down the flank and over the rump. It will be noticed that the hair replacement on the limbs appears to occur independently of the dorso-lateral cycle.

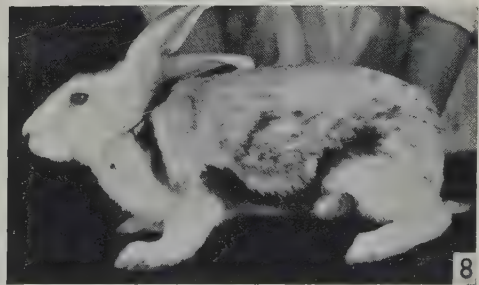
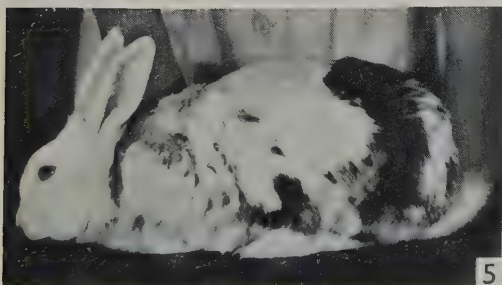
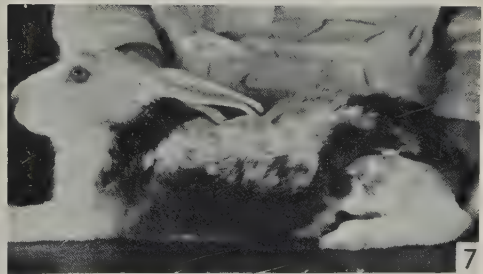
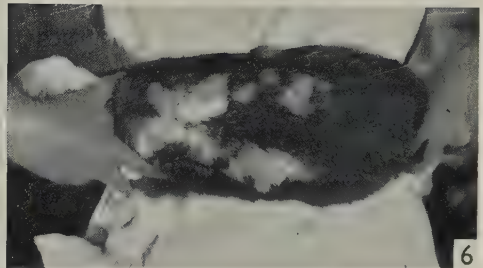
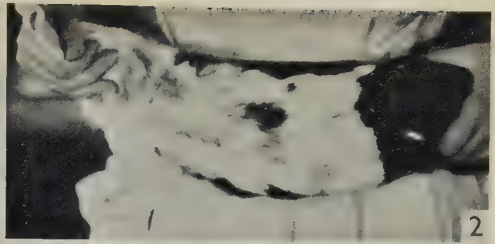
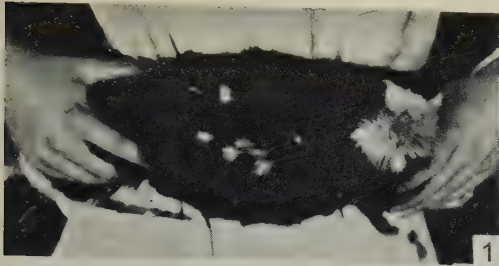
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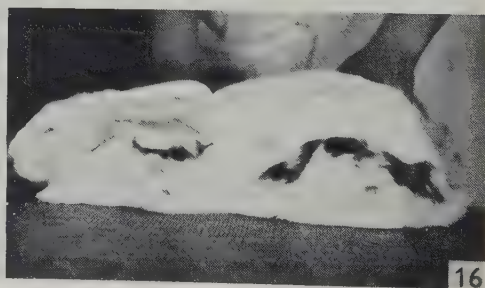
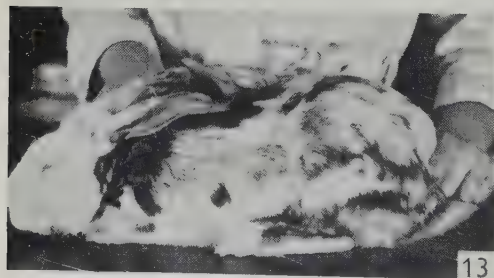
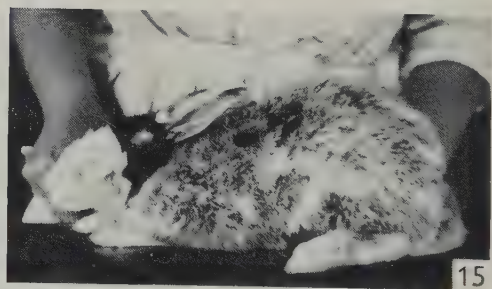
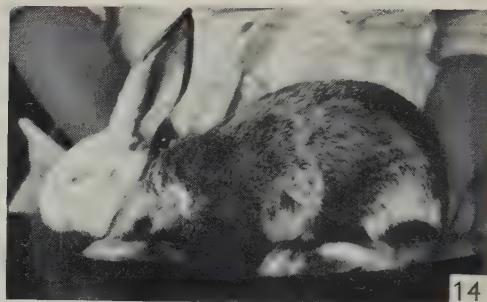
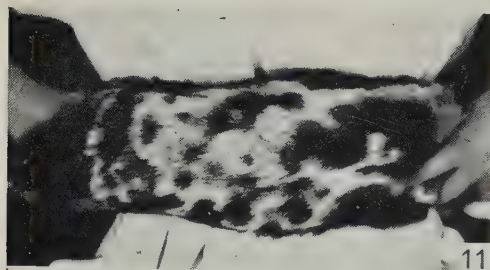
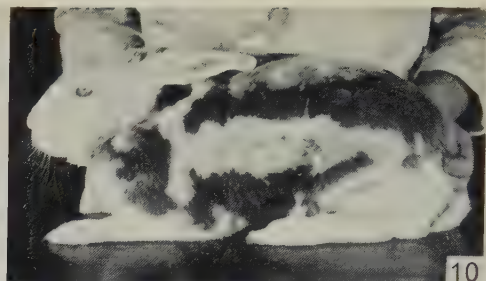
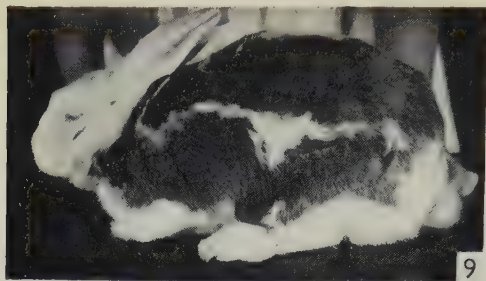
- Fig. 9. Adult albino rabbit (no. 71). Lateral view showing a linear lateral band of replacement. This was visible soon after the initial dyeing and is due to the dyeing of the animal during the course of the lateral progression of the hair replacement cycle.
- Fig. 10. Rabbit (no. 71). Lateral view showing ventral progression of the cycle and independent replacement on the limbs.
- Fig. 11. Adult albino rabbit (no. 75). Dorsal view showing the complicated dorsal initial pattern sometimes seen.
- Fig. 12. Rabbit (no. 75). Lateral view after a period of 5 weeks showing lateral spread of the dorso-lateral cycle.
- Fig. 13. Rabbit (no. 75). Lateral view after a further period of 6 weeks showing further downwards spread of the dorso-lateral cycle.
- Fig. 14. Young albino rabbit (no. 72) showing appearance 4 weeks after the initial dyeing. The appearance is different from the adult in that the replacement is not complete, the newly grown white hairs being added to the existing dyed hair and the zone of growth is larger and more diffuse.
- Fig. 15. Young albino rabbit (no. 30). Ten weeks after initial dyeing showing abundant growth of new hair diffusely over the trunk.
- Fig. 16. Rabbit (no. 30). Thirteen months later almost at the completion of a further cycle, now showing the adult pattern, the replacement being complete.



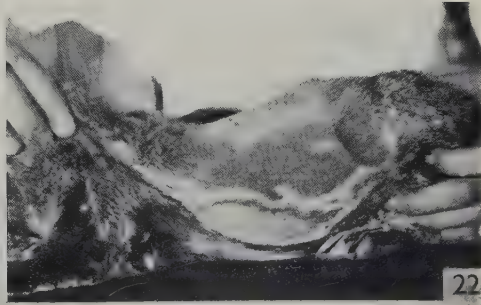
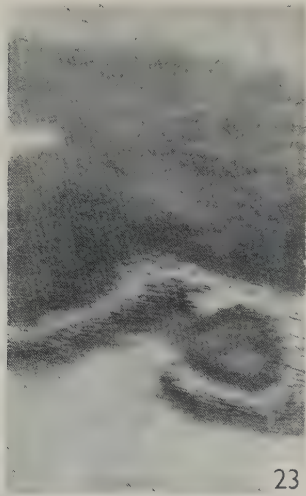
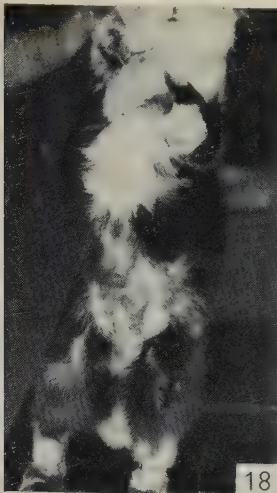
## PLATE 3

- Fig. 17. Adult albino rabbit (no. 57) showing the ventral replacement of hair. There is the beginning of replacement in the centre after an initial quiescent period of 8 weeks.
- Fig. 18. Rabbit (no. 57) after a period of 8 weeks.
- Fig. 19. Rabbit (no. 57) after a further period of 10 weeks.
- Fig. 20. Rabbit (no. 57) showing the completed replacement of the ventral hair after a total period of 6 months. During this time there was no replacement of the hair on the dorsum or flanks.
- Fig. 21. Dorsal view of a clipped chinchilla rabbit showing the dorsal zone of hair growth. The growing zone is thick and pigmented, while the remaining skin is pink.
- Fig. 22. Lateral view of a clipped chinchilla rabbit showing the characteristic appearance of the lateral band of hair growth.
- Fig. 23. The lateral band of hair growth in detail in the clipped animal showing a narrow pigmented ventral edge, a dark zone with shafts, a white zone with shafts and a wide dark zone fading to grey.











## THE LEVATORES COSTARUM AND THEIR NERVE SUPPLY

By A. B. MORRISON

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The most detailed account of the levatores costarum and their innervation is to be found in the section on myology in Bardeleben's *Anatomie des Menschen*, which was written by Eisler (1912); he describes fine branches of each intercostal nerve which pass posteriorly through the posterior intercostal membrane to enter the anterior surface of the respective levator costae muscle. It is his description that is followed in the most recent edition of Quain and the current British text-books of anatomy.

In assessing the morphology of the post-vertebral and other small muscles of the back much weight has necessarily been laid on their innervation. On this account the levatores costarum have been classified usually with the ventro-lateral musculature (Howell, 1936; Cave, 1937), as the innervation is assumed to be from the intercostal nerves. Dissection in the human subject and in animals indicates the inaccuracy of the standard descriptions of the nerve supply of these muscles. For this reason an account of the innervation of the levatores costarum muscles, as revealed by studies in man and certain mammals, is given below.

### METHODS

Special dissections were made for four human cadavers from the posterior aspect, to facilitate examination of the levatores costarum muscles from their medial aspects. After reflecting the skin of the back, the medial and lateral branches of the posterior-primary rami were picked up and retained while the back muscles were removed, special care being taken as the muscles of the sacrospinalis mass were being stripped. The spinal canal was opened by nibbling away the laminae and exposing the roots of the spinal nerves. These were followed to their corresponding dorsal root ganglia and through the intervertebral foramina. The division of each nerve into its posterior and anterior ramus was verified and the posterior ramus was then traced posteriorly between the vertebral body, superior costo-transverse ligament and the point where the pedicle joins the lamina. Here the nerve lies antero-medial to the corresponding levator costae and it may be followed posteriorly, medial to the levator, to divide into its medial and lateral divisions, the lateral division passing between the intertransverse ligament and the medial border of the corresponding rib, while the medial division passes medial to the intertransverse ligament. This part of the dissection is shown in Fig. 1.

To expose the course of the nerve more clearly the transverse process was cut through where it joins the pedicle. This operation was more easily accomplished with the prior removal of the lamina. The posterior costo-transverse ligament was now cut and the transverse process with the corresponding levator costae arising from it was gently turned down, away from the facet of the rib with which it articulated,

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and the superior costo-transverse ligament attached to its inferior border was thus exposed and cut through. This permitted the levator costae muscle to be turned laterally and the structures lying medial to it to be more easily examined. This is shown in Fig. 2.

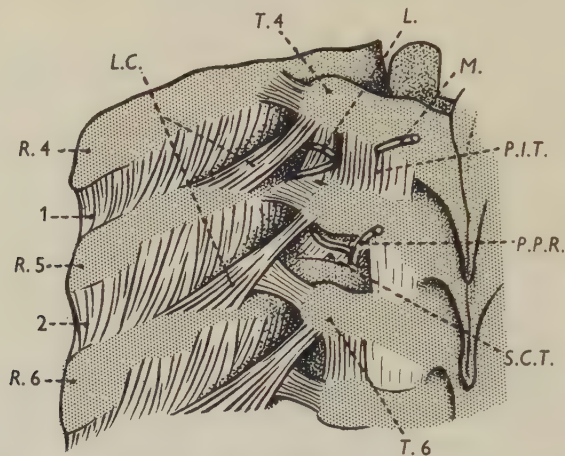


Fig. 1. The relationships at the posterior part of the intercostal spaces. In space (1) the medial (M.) and lateral (L.) divisions of the posterior primary rami are seen issuing on either side of the posterior intertransverse muscles (P.I.T.). In space (2) the intertransverse muscle has been removed to expose the posterior primary ramus (P.P.R.) issuing medial to the superior costo-transverse ligament (S.C.T.) and dividing into its two divisions. R. 4, R. 5, R. 6, the 4th, 5th and 6th ribs; T. 4, T. 6, transverse processes of the 4th and 6th vertebrae, respectively; L.C., levator costae.

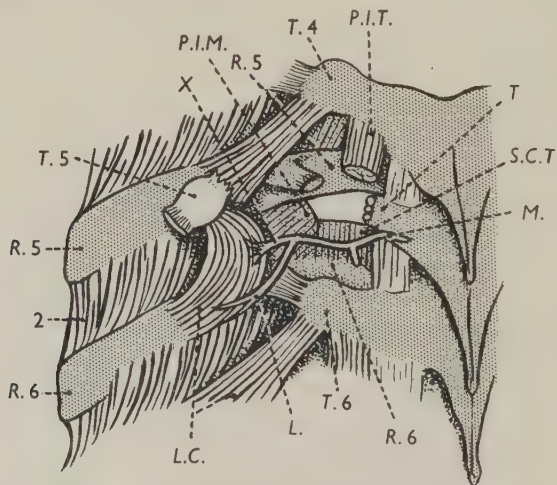


Fig. 2. The posterior part of the intercostal space (2) between the 5th and 6th ribs. The transverse process (T. 5) has been removed as described in the text, and it and the attached levator costae have been pulled laterally exposing the superior costo-transverse ligament which was cut through where it was attached to the transverse process before the process could be removed. The posterior intercostal membrane (P.I.M.) can also be seen fusing medially with the superior costo-transverse ligament. The branch of the lateral division of the posterior primary ramus entering the levator costae can be easily seen (X).

A special dissection of the 8th cervical nerve and its posterior primary ramus was carried out in each cadaver. The intercostal nerves were carefully dissected from the inner aspect of the thorax to discover the branches of the intercostal nerves that are said to pass to the levatores costarum. In addition to gross dissection several blocks of tissue consisting of a levator costae muscle and the nerve to it were dissected using a dissecting microscope. Similar blocks of tissue comprising the whole thickness of the thoracic wall in one intercostal space, and the corresponding posterior primary ramus and its branch to the levator muscle were embedded, sectioned and stained with haematoxylin and eosin to demonstrate the ramifications of the nerves in the muscles.

An examination of the innervation of the levatores costarum in the guinea-pig and in the rat was made, using Sihler's (1900) technique. The posterior part of the thorax of a rat, together with its vertebral column, was stripped of the muscles attached to it except the levatores costarum and the intercostals and a few small shreds of muscle over the laminae. The whole mass was then placed in Sihler's solution A for 24 hr. and then in solution B. Fairly good results were obtained, the muscles being almost transparent and the nerves standing out in transmitted light. The nerves seem to stand out because of some inherent refractive property rather than being more selectively stained.

A dissection of the back muscles of a dog and a cat was also made.

#### RESULTS

In the human, the levatores costarum were found, without exception, to be supplied by a branch of the posterior primary ramus of the corresponding spinal nerve. The branch arises in most cases from the lateral division but in one intercostal space in one cadaver it arose from the ramus before it divided into medial and lateral divisions. The branch to the levator costae muscle in each space emerged in the interval between the medial border of the muscle and the intertransverse ligament in association with the lateral division of the posterior primary ramus and lying on its lateral side. The branch to the muscle coursed laterally for a short distance over the muscle and entered it on its dorso-medial surface, closer to its insertion on the rib than to its point of origin. The ramifications of the nerve in the muscle were followed as described, using the dissecting microscope.

That the tissue being followed through the muscle was in fact nervous tissue, and not some strands of fibrous tissue mistaken for it, was confirmed by examination of the sections made from the tissue blocks obtained, as described above. The ramifications of the nerve in the muscle were followed in serial section and it could be seen that the nerve broke up and sent branches through every part of the muscle. On these same sections the posterior intercostal membrane was examined and at no point were any nerve fibres observed passing through it.

The 8th cervical nerve was dissected and a branch was observed to issue from it immediately after it emerged from the ganglion. This nerve passed posteriorly into the cleft between the 1st levator costae muscle and the last posterior cervical intertransverse muscle which lay immediately medial to it. Turning round the medial side of the levator muscle it sent a large branch into the dorso-medial surface.

In the rat the posterior primary ramus gave off a branch just before it divided

into medial and lateral divisions and this branch passed laterally into the levator costae muscle and the ramifications were followed in the muscle for quite a distance. A similar state of affairs was found in the guinea-pig.

In the dog and in the cat the levatores costarum muscles were dissected and the nerve of supply from the posterior primary ramus was also demonstrated.

#### DISCUSSION

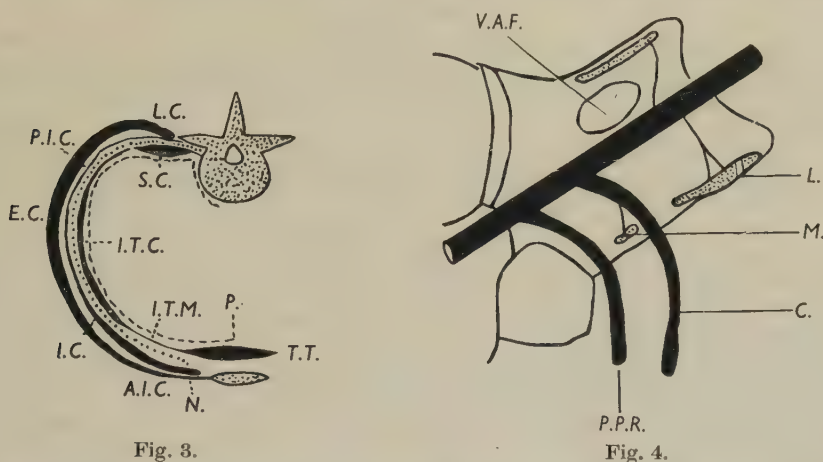
In his account of the muscular system (written in K. von Bardeleben's *Anatomie des Menschen*), Eisler (1912) describes each levator costae as being supplied by fine branches from the intercostal nerve, the branches passing through the posterior intercostal membrane and the posterior part of the external intercostal muscle. This account of the nerve supply has been followed and accepted up to the present time. Bryce (1923) follows Eisler, and in all the current texts the levatores costarum are still described as being supplied by the anterior primary ramus of the corresponding spinal nerve. Long before Eisler's account was published it is interesting to note that in Owen's *Comparative Anatomy of Vertebrates* (1868) a description of the anatomy of the back muscles of the porpoise includes the statement that the levatores were supplied by the lateral divisions of the posterior rami.

In accord with the nerve supply from the intercostals it has generally been accepted that the levatores costarum belong to the ventro-lateral musculature. Walmsley (1916), in his account of the intercostal musculature, places the levatores along with the external intercostals saying that they are continuations posteriorly of the outer layer of the ventro-lateral musculature. His account and accompanying diagram (see Fig. 3), are interesting in that he pictures the levatores as being in the same plane as the external intercostals and implies this in the text. However, careful examination will convince anyone that the levatores costarum overlap the posterior parts of the intercostals and that the whole muscle bundle lies on a more superficial plane than the external intercostals. In a morphological classification of the vertebral muscular system, Howell (1936) places the levatores costarum with the ventro-lateral musculature.

Cave (1937), in his description of the cervical intertransverse muscles, regards the levatores costarum as supplied by the anterior primary ramus and attempts to homologize them with the lateral division of the lumbar intertransverse musculature also supplied by the anterior primary ramus, and maintains that they have no corresponding element in the cervical region. He does this on the basis of the nerve supply and the origin and insertion of the levatores. They arise from the diapophysis and insert on the pleurapophysis and according to him, since they have a nerve supply from the anterior primary rami, must be analogous to the lateral group of the lumbar intertransverse. The fact that the levatores are supplied by the posterior primary rami invalidates this; indeed this nerve supply suggests that the levatores costarum are homologous with the lateral parts of the proper posterior intertransverse muscles of the cervical region. It will be recalled that there are two posterior intertransverse muscles in the cervical region; one, the lateral attached to the posterior tubercles of the transverse processes and supplied by the anterior primary ramus, and the other medial attached to the posterior bar of the transverse process and supplied by the posterior primary ramus. This is shown in Fig. 4.



The medial muscle itself is divided into two parts, a medial and a lateral, by the lateral division of the posterior primary ramus, according to Eisler; and Cave has described the same state of affairs in two out of six dissections. It is suggested here that the levatores costarum are homologous with the lateral of the two divisions of the proper posterior intertransverse muscle of the cervical region, while the posterior intertransverse muscle of the thoracic region, which is usually reduced to a ligament in the middle of the thoracic region, represents the medial division of the proper



posterior intertransverse muscle of the cervical region. The separation of the levator costae and the intertransverse muscle or ligament in the thoracic region by the lateral division of the posterior primary ramus may be compared to the division of the proper posterior intertransverse muscle into two parts in the cervical region by the lateral division of the posterior primary ramus.

That this is indeed true is suggested by the nerve supply of the 1st levator costae where the posterior ramus of the 8th cervical nerve issues between, and supplies, both the last posterior intertransverse muscle and the first levator costae. At this place the two muscles are usually indistinguishable from one another.

In the lumbar region the levatores costarum are represented by the medial posterior intertransverse muscles and not by the lateral posterior intertransverse muscles, as was suggested by Cave. It would seem that these latter muscles must be homologous with the intercostal muscles. Since they arise from the accessory processes above and insert on the transverse processes below, it can be surmised that the accessory processes may contain a pleurapophyseal element as well as a diapophyseal element, as required by the origin from them of the medial posterior intertransverse muscles. Though they arise from transverse processes the insertion of the

levatores costarum on to the ribs is difficult to understand if they are homologous with the posterior intertransverse musculature. However, it must be assumed that their insertions have migrated laterally though they have retained their original nerve supply. The highest of these muscles is frequently fused with, and difficult to distinguish from, the last cervical intertransverse and possibly represents the relationship of the levatores to the other muscles before such migration had taken place.

#### SUMMARY

The levatores costarum are supplied by the lateral divisions of the posterior primary rami of the corresponding spinal nerves and not by the intercostal nerves as is currently accepted.

It is suggested that the levatores costarum muscles correspond to the lateral portion of the proper posterior intertransverse muscles of the cervical region.

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# THE MECHANICS OF THE FOOT

## II. THE PLANTAR APONEUROSIS AND THE ARCH

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*Birmingham*

### INTRODUCTION

In a normal living foot that is weight-bearing, as in ordinary standing, passive extension of the big toe at the metatarso-phalangeal joint will be observed to result in the following effects:

- (i) the arch appears to rise (Pl. 1, figs. 1, 2);
- (ii) the posterior part of the foot assumes an 'inverted' position (supinates);
- (iii) the leg rotates laterally;
- (iv) there appears a tight band in the region of the plantar aponeurosis (Pl. 1, figs. 2, 3).

It has been explained in a previous paper (Hicks, 1953) that (i) is synonymous with flexion of the first ray. The terms 'flexion of the ray', 'rising of the arch' and 'downward movement of the metatarsal head' will therefore be used alternatively throughout the present paper. It was also explained that (ii) and (iii) occur whenever individual flexion of the first ray—and in consequence pronation twist of the forefoot—takes place in the standing foot. The short terms 'flexion' and 'extension' are being used for a movement which has previously been shown to be really flexion-pronation extension-supination. The present discussion is not invalidated by this approximation.

When the load is light or when it is being borne mostly on the heel, toe extension to its limit (about 90° to the line of the metatarsal) can be brought about with comparatively little force, and in these circumstances the subject's own extensor hallucis longus muscle will succeed in bringing about the action. A few trials will reveal, however, that as a greater proportion of body weight falls on to the anterior part of the foot the toe extension requires greater force, the extensor muscle becomes inadequate, and the subject experiences an almost painful feeling of tightness in the sole. At this stage he tends to invert his foot voluntarily to relieve this painful sensation. These effects, however, also occur in the dead or the paralysed foot, so it is evident that the action of the inverter muscles of the foot is not necessary.

The four observations listed above also hold in toe-standing (Pl. 1, fig. 3), the metatarso-phalangeal joints in this case being caused to extend by pressure of the toes against the ground. This is a familiar test in the clinical examination of the foot, the usual, though erroneous, interpretation being that the arch-raising muscles have thereby been proved to be working satisfactorily.

When the foot is free, the arch-rising effect is manifested as a movement of the metatarsal head downward relative to the fixed posterior part of the foot. An attempt to oppose this will reveal that—so long as metatarso-phalangeal extension is achieved—the downward shift of the metatarsal head is irresistible. It is demonstrable in lesser degree in the second, third and fourth rays, a prominence of the



metatarsal head in the ball of the foot being the manifestation of its downward shift relative to the rest of the foot. This prominence disappears and the metatarsal head recedes into line with its neighbours when the toe is released and allowed to flex. The effect is almost absent in the fifth ray.

Although the association of arch-rising with toe-extending is recognised by orthopaedic surgeons no attempt has been made to explain it and its existence receives no more than an incidental mention in the literature (Jack, 1953). A short version of the present work has appeared previously (Hicks, 1951).

In the standard anatomical text-books no reference is made to the plantar aponeurosis as an agent in raising the arch, although several text-books (Buchanan (1949), Cunningham (1951), Gray (1949)) include it amongst other structures, e.g. plantar ligaments and muscles, which maintain the arch, likening it to the string of a bow. Concerning the anterior attachment of this bow-string there is general agreement that, having divided into five digital processes, the main part of the plantar aponeurosis becomes attached to the sesamoid bones, the deep transverse ligament of the sole and the fibrous sheaths of the flexor tendons and hence to the proximal phalanges. The importance of the mechanical effect of the attachment to the phalanges will be explained in this paper.

#### MATERIAL AND METHODS

The material described by Hicks (1953) was used.

I. The effects of extending the toes at the metatarso-phalangeal joints were observed in the experimental foot in the natural conditions of standing.

II. Dissection was carried out to identify the tight band observed in the sole and to examine its mechanical effects. This involved a study of the plantar aponeurosis and especially of its anterior attachments. The aponeurosis was ultimately divided to determine whether or not it was essential to the arch-raising mechanism.

III. Before dissection had proceeded far enough to interfere with the mechanism radiographic examination was made to demonstrate it in action. The aponeurosis, the process going toward the toe, and the plantar pad of the metatarso-phalangeal joint were marked by metal clips. Radiographs, both with toes flexed and toes extended, were taken using techniques designed to demonstrate changes in arch height.

IV. Analysis of the metatarsal movement was made by the method of the artificial axis (Hicks, 1953).

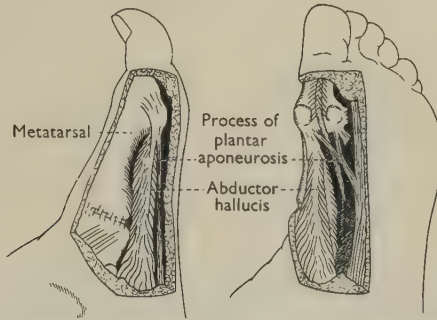
V. The living foot was examined in action by cinematography and radiography, and the movements occurring at the heel-raising phase of walking were identified with those observed in the specimen.

#### OBSERVATIONS AND INTERPRETATIONS

I. All the effects described on p. 25 in the living foot were observed to occur in the specimen. It was concluded, therefore, that the mechanism is independent of muscles.

II. Dissection revealed that two structures play an important part functionally. They are the plantar pads of the metatarso-phalangeal joints and the plantar

aponeurosis which is attached to them through its five digital processes. The plantar pad is the thick conjoined tendon and capsule on the plantar aspect of each joint. The name is adopted from a paper by Haines (1947), although, as will presently become clear, it is not fully appropriate, suggesting as it does the function of a cushion rather than that of a cable. Each plantar pad with its attached process of plantar aponeurosis was seen to constitute a continuous strong band forming a direct connexion between the proximal phalanx and the calcaneum like a tie or bow-string. Each tie was observed to become tense when an attempt was made to push the corresponding metatarsal head upwards and all became tense in standing. This tension appeared just as the ray reached its limit of extension, which is to say when



Text-fig. 1. Semi-diagrammatic drawings of a dissection of the arch-raising mechanism.

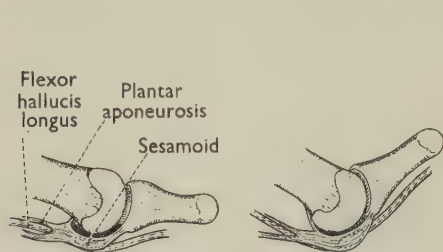
the arch reached its limit of flattening. If the toe to which it was attached was first held in extension, the tie was observed to become tense at an earlier stage, i.e. it prevented the ray from reaching its usual limit of extension. Alternatively, if the ray was initially pushed into the fully extended position and then the metatarsophalangeal joint was extended, the ray became forcibly flexed, being pulled into flexion by a progressive shortening of the tie.

To explain these phenomena the following details of structural anatomy are necessary. Although made independently they are in close conformity with the description given by Haines whose other observations are also fully confirmed.

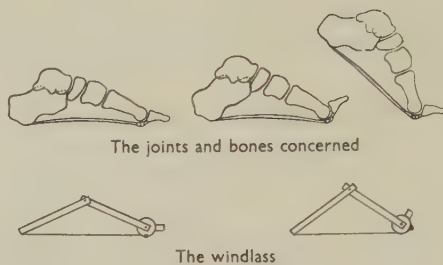
The five strong processes into which the plantar aponeurosis divides, each split into two slips which straddle the toe flexor tendons (Text-fig. 1). The larger part of each slip fuses with the plantar pad near to the sesamoid thickening. The insertions were of substantial size in the specimens quoted (though less obvious in certain senile feet), being comparable with the adjacent insertions of the short muscles. The plantar pad (Text-fig. 2) is broad and thick. It is connected very firmly to the base of the proximal phalanx, not only to the lower rim of the articular facet but also blending with the periosteum on the inferior surface. Its superior aspect is an articular surface continuous with that of the proximal end of the phalanx and forming two-thirds of the total surface for articulation with the metatarsal head. It moves with the phalanx, being free to slide anteriorly and posteriorly underneath the metatarsal head. At the same time the toe flexor tendons slide equally freely underneath the plantar pad and have no direct mechanical action upon the

mechanism. The part of the joint capsule attached to the neck of the metatarsal and closing the joint cavity proximally is lax and tenuous allowing of free movement but having little mechanical strength.

Haines has pointed out a continuity of ligamentous tissue over the surface and around the sides of the sesamoids, and it is apparent that together with the plantar aponeurosis the plantar pads provide a continuous band of ligamentous tissue between calcaneum and proximal phalanges suitable to the transmission of a mechanical pull to or from the phalanges.



Text-fig. 2. Mechanical detail at the metatarso-phalangeal joint.



Text-fig. 3. The windlass.

The toe-extending arch-raising effect was seen to occur as follows. When the toe was extended, the phalanx, sliding on to the dorsum of the metatarsal head, pulled after it the plantar pad which thereby came to lie anterior to the metatarsal head and this in turn pulled upon the attached process of the plantar aponeurosis. The effect was as though a cable had been wound one-quarter of a turn on to the drum of a windlass (Text-fig. 3; Pl. 1, figs. 4, 5), the drum of the windlass being the head of the metatarsal, the handle which does the winding being the proximal phalanx and the cable which is wound on to the drum being the plantar pad and the plantar aponeurosis. The effective length of the cable was shortened by, in the case of the first ray, about 1 cm. Actually the aponeurosis did not shift distally because of its attachment to the calcaneum; instead it was the windlass which shifted, being pulled 1 cm. proximally towards the calcaneum (cf. Text-fig. 4) and the arch was thereby made shorter and higher. When one toe alone was extended the corresponding metatarsal head was found to be moved by the mechanism posteriorly and downwards out of line with its neighbours in the ball of the foot and to remain there as a prominence resistant to all pressure until the metatarso-phalangeal joint was allowed to flex again.

To confirm that the 'cable' is strong enough to perform this function, tests were made in four specimens outside the series. It was found that the total breaking strain of the mechanism to the five toes ranged from  $1.7 \times$  to  $3.4 \times$  body weight.

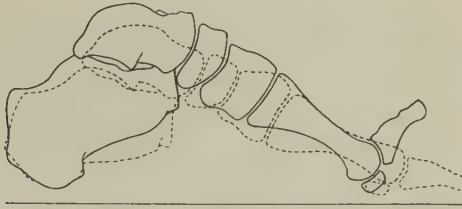
When the plantar aponeurosis was cut across, the arch-raising action almost disappeared.

No muscle is directly concerned in the mechanism which is entirely bony and ligamentous, the work of raising the arch being done usually by body weight. The action is analogous to the 'tendon action' of muscles that cross two joints described by Elftman (1939).



III. The radiographs (Pl. 1, figs. 4, 5) demonstrate this flexion of the ray which results when the toe is extended, the metal clips showing up the windlass effect. Radiographs of the living foot revealed similar effects, and tracings from these, superimposed (Text-fig. 4), demonstrate the approximation of metatarsal head to calcaneum, also the ray flexion or increase in height of the arch. The sesamoid shadow acts as a guide to the position of the plantar pad as the metal clips do in the experimental foot.

IV. The measured range of movement as brought about by the windlass varied in different specimens but the findings averaged about half of the full range of which the joints were capable, being about  $10^{\circ}$  for the first ray (full range =  $22^{\circ}$ ) and about  $5^{\circ}$  for the lateral rays (full range =  $10^{\circ}$ ). This range was from a position of full extension to one of semi-flexion. The movement took place at the naviculo-cunei-



Text-fig. 4. Tracings from radiographs of a living foot, standing. Dotted line: toe-flexed low-arch position. Continuous line: toe-extended high-arch position.

form and cuneo-metatarsal articulations and movement at other articulations did not occur when the foot was free. (The movement at the talo-navicular articulation, which can be detected in the tracings (Text-fig. 4), is a manifestation of talo-calcaneo-navicular joint movement, viz. supination-adduction-flexion ('inversion') which is one of the secondary movements that occur when the foot is standing.)

Although this mechanism exists in each of the five rays its effects are most marked in the first ray. Thus, when all the toes are equally extended, the first ray shows the greatest degree of flexion and the fifth the least. This is pronation twist of the forefoot (Hicks, 1953). The consequences of fixed pronation twist of the forefoot when standing have been described in the previous paper as supination of the posterior part of the foot and lateral rotation of the leg.

V. The mechanism functions each time a step is taken. During the phase when the foot is rising on to the toes the toes are being extended by pressure against the floor. A cinematograph film was made of the foot of a person walking, and it was seen that the arch does rise during this phase. The effect of the ray flexion in walking is to increase the range and speed of flexion over and above that which occurs at the ankle alone and to provide a foot which does not yield to the increasing forces at the toe-rising phase but which tends to flex and thrust downwards with an additional 'flick' on taking off. The new concept which emerges is that the arch-raising is not necessarily the result of action by arch-raising muscles but is a movement that must inevitably occur in every foot, even if dead or paralytic, every time the toes are

extended. This, however, must not be taken to imply that muscles never have an action upon the arch.

The mechanism should also work in reverse, i.e. the effect of body weight should tend to flatten out a raised arch, the flattening of the arch should tend to unwind the windlass, and the unwinding of the windlass should tend to flex the toe at the metatarso-phalangeal joint. Experiment showed this to be the case. When the foot, dead or living, is made to bear weight the toes are found to press upon the ground by a flexion action arising at the metatarso-phalangeal joints. This pressure, in the experimental specimen, was found to vary with body weight. The effect disappeared when the plantar aponeurosis was divided. It can be concluded, therefore, that part at least of the 'gripping action' of toes on ground so often referred to in discussions on walking is not due to the action of the toe flexor muscles.

#### SUMMARY

1. The plantar aponeurosis at its distal end is attached through the plantar pads of the metatarso-phalangeal joints to the proximal phalanges. The attachment is mechanically very strong.

2. When the toes are extended they pull the plantar pads and hence the aponeurosis forward around the heads of the metatarsals, like a cable being wound on to a windlass. The arch is caused to rise because the distance between the metatarsal heads and the calcaneum is thereby shortened.

3. The toes are forced into an extended position in toe-standing and walking by the action of body weight, and the arch is caused to rise by this ligamentous mechanism without the direct action of any muscle.

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#### EXPLANATION OF PLATE 1

Figs. 1, 2, and 3. Normal living foot.

Fig. 1. Relaxed flat standing. The arch is at the lower limit of normal range.

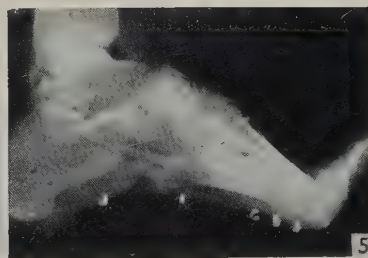
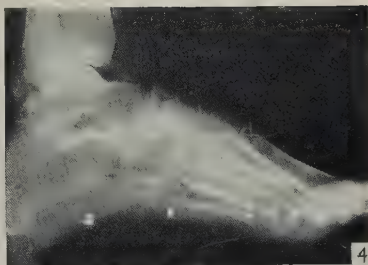
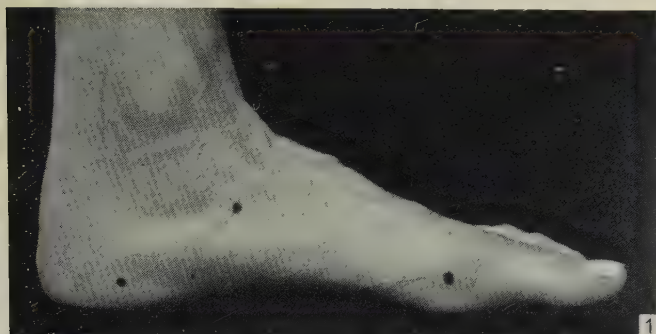
Fig. 2. Extension of the toe causing the arch to rise.

Fig. 3. The same effect occurring in toe-standing. The tight band of the plantar aponeurosis is seen in the sole.

Figs. 4, 5. Radiographs of the windlass mechanism in the experimental foot. Metal clips have been fixed to the plantar aponeurosis, to its process going towards the big toe and to the plantar pad of the metatarso-phalangeal joint. (The upward convexity of the main part of the aponeurosis is due to the intermuscular septum attached deeply.)

Fig. 4. Toe-flexed low-arch position.

Fig. 5. Toe-extended high-arch position.







# STUDIES ON THE CULTIVATION OF TEETH IN VITRO

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## INTRODUCTION

The purpose of the work described in this paper was to clarify certain problems of the functional histology of teeth by the use of tissue-culture technique. Beyond the pioneer work of Glasstone (1936, 1938, 1952), who showed that teeth are self-differentiating organs and capable of regeneration and repair *in vitro*, tissue culture has been little used for this purpose; and only preliminary reports have been published by other investigators (Nuckolls, 1941*a* and *b*; Loose, 1943).

The particular problems studied in the present paper are: (a) the study of the cellular composition of teeth and the growth characteristics of its various cells *in vitro*; and (b) the study of how teeth as organs react to different environments *in vitro*.

(a) The analysis of cellular composition was investigated by studying the outgrowth from hanging-drop cultures, the method best adapted to the purpose. In hanging-drop cultures a certain anatomical disorganization of tissues takes place, and cells from the cultivated fragment grow out into the plasma coagulum that forms the culture medium. Although the cells are very much altered under conditions of free growth, it is nevertheless true to say that cells of different types preserve quite characteristic distinctions of shape, habit of growth and cell-interrelationship *in vitro*.

Two cell types may be of interest in this respect: odontoblasts and the cells of the enamel organ. Odontoblasts possess special characteristics of both mesenchymal and epithelial cells, being originally located in the dental papilla and physiologically related to osteoblasts and yet, when fully differentiated, often assuming the shape and arrangement of a cuboidal or cylindrical epithelium with the long axis of the cell perpendicular to the dentine layer. It will be seen that these various peculiarities of odontoblasts are reflected in their structure and habit of growth *in vitro*.

(b) Two alternative methods were used in order to study the behaviour of teeth as organized tissue systems in different environments. Organized growth occurs within the central explants of hanging-drop cultures, and, as in Glasstone's work, can be revealed by orthodox histological sectioning. A more suitable method, however, is the cultivation of explants in *fluid* media under conditions allowing functional survival without outgrowths of cells (cf. Parker, 1936; Medawar, 1948). Cultures in fluid media can be studied only by ordinary histological means.

The work described here is of a preliminary nature. An attempt has been made to correlate findings *in vitro* with the evidence of experimental dental physiology, in which it is hoped that the technique of tissue culture may find a useful place.

## METHODS

*Materials*

Strong-A-line albino mice (see Strong, 1942) were used throughout the experiments, except in one series, where teeth of albino rats were cultured. Molars or incisors, erupted or unerupted, were explanted. Dissections were performed under aseptic conditions and streptomycin was used as an antibiotic, an aqueous solution containing 500 units per ml. being diluted before use with nine times its volume of the culture medium (Medawar, 1948).

The corresponding tooth from the opposite side was fixed as a control, or in some rocker flask experiments incisors of litter-mates were used as controls.

Before the beginning of the investigation of the cellular composition of the outgrowth in hanging-drop cultures, tests were carried out with the aim of finding a suitable material which would certainly contain odontoblasts or the enamel organ and would preserve its normal shape, thus making orientation possible *in vitro*. The following tissues or organs were tested:

- (1) Unerupted or erupted molars or incisors, cultured as a whole.
- (2) Extirpated pulp from unerupted molars.
- (3) Enamel organ of unerupted molars.
- (4) Extirpated pulp and growing end of incisors, cultured together in the same hanging drop.
- (5) Extirpated pulp of incisors cultured in isolation.
- (6) Dissected growing end of an incisor in isolation.
- (7) Dissected enamel organ of an incisor in isolation.
- (8) Periodontal membrane of an incisor in isolation.
- (9) The remaining hard tissue of an incisor (dentine, enamel matrix, and enamel).

To facilitate orientation *in vitro*, the explants were disposed in such a way that the long axis of the incisors and the lingual surface of the molars were parallel to the cover-glass. It was thus possible to distinguish between the crown and apical area of a molar or between the tip and the growing end of an incisor. The extirpated pulp of an incisor is rather rigid, preserving the original curvature of the tooth, so that orientation *in vitro* was easy. The enamel organ is very easily distorted, making orientation difficult *in vitro*; for this reason parts of the enamel matrix were left on the distal part of the organ for ease of identification.

In cultures in fluid media whole incisors, together with the alveolus, were explanted, and only a small opening was cut through the alveolar socket in order to establish an immediate contact between the tooth itself and the culture medium.

*Culture vessels and media*

Orthodox hanging-drop cultures and cultures in rocker flasks (Medawar, 1948) were employed; to avoid degeneration of the outgrowth and its inevitable disturbance during subculturing, Maximow's double cover-slip method was also used in certain experiments (Maximow, 1925).

In the hanging-drop experiments culture media consisted of chicken plasma and an extract of minced 11 to 14-day-old chicken embryos. As diluting fluid Pannett and Compton phosphate-buffered saline (Pannett & Compton, 1924) was employed.



In the rocker flask cultures parallel experiments were carried out in the following solutions: (a) embryo extract 2 ml., 5.3 % glucose 1 ml. streptomycin (500 units/ml. 1 ml., and Pannett & Compton saline 6 ml.; (b) embryo extract 2 ml., streptomycin (500 units/ml.) 1 ml., rabbit serum, 7 ml.

The orthodox hanging-drop cultures were not subcultured for fear of disturbing the outgrowth. They were therefore kept not longer than a week. The cultures made by the double cover-slip method were washed every 3–4 days, and the fluid medium cultures were washed and transferred into new culture flasks after the same intervals.

In a few flask cultures the effect of a relatively high oxygen tension was also studied, by perfusing the flasks with the mixture: O<sub>2</sub> 60 %, N<sub>2</sub> 39 %, CO<sub>2</sub> 1 %.

### *Histological technique*

For histological examination the whole cultures or explants were fixed in 4 % formol-saline and were either stained and mounted as a whole (to study the outgrowth) or were embedded in paraffin wax and sectioned. The special embedding method for teeth recommended by Manley & Brain (1949) was used. The sections or the whole mounts on the cover-slips were stained with Harris's haematoxylin and eosin. The sections were also stained with Weigert's haematoxylin and van Gieson's picrofuchsin or by Mallory's connective tissue stain.

## RESULTS

The observations are based on hanging-drop cultures (orthodox or double cover-slip) of 142 molars and 90 incisors. Fifty-four incisors were cultured in rocker flasks.

### SECTION I. HANGING-DROP CULTURES

#### *A. Gross observations on the outgrowth*

##### *(a) Short description of development and histology of teeth*

It may be useful at this point to give a short description of the development and histology of teeth (Pl. 1, figs. 1–3). A tooth germ (Pl. 1, fig. 1) comprises the enamel organ, a derivative of the oral epithelium and the dental papilla, deriving from the underlying mesenchyme. Both are responsible for the formation of 'hard' tissues, viz. enamel and dentine respectively.

In the early stages of development of a tooth germ, the enamel epithelium consists of four layers: outer enamel epithelium, stellate reticulum, stratum intermedium and inner enamel epithelium. The inner enamel develops later into ameloblasts, which are responsible for the deposition of enamel matrix and for its maturation into enamel (see Marsland, 1952). During this process the enamel organ undergoes reduction in size and at the end of maturation disappears completely thus leaving the enamel of an erupted tooth exposed.

It is generally assumed that, in mammals at least, the enamel organ 'induces' the formation of odontoblasts. This role is performed in developing roots by the epithelial sheet of Hertwig, a downgrowth of the enamel organ (Pl. 1, fig. 3).

The dental papilla develops into the dental pulp, at first covered by predentine or dentine matrix which after calcification becomes transformed into dentine. Next

to the predentine is the odontoblastic layer (Pl. 1, fig. 2, *E*). The pulp also contains fibroblasts, blood vessels, nerve fibres, etc.

As Schour & Ham (1934, p. 26) pointed out: 'Even in normal dentine the successive layers are not equally well calcified. Well-calcified layers alternate more or less regularly and rhythmically with imperfectly calcified layers, so that there arises a stratification'. The dentine therefore, especially of persistently growing incisors, is a delicate chronological record of changes in calcification. This growth-ring pattern can be demonstrated in decalcified sections, where the calcified matrix of dentine takes haematoxylin, the uncalcified matrix takes eosin stain (see Schour & Ham 1934). 'Calciotraumatic line' of Schour (Thoma, 1950) is the result of a sudden change in the rate of calcification, e.g. after parathyroidectomy.

The dentine of the root of a tooth is covered by cementum and surrounded by the periodontal membrane.

The incisors of rodents (Pl. 1, fig. 2) are persistently growing teeth, and are covered with enamel only on their convex, labial side. There is an enamel organ on the lingual side as well, but having 'induced' dentine formation, it degenerates without depositing enamel matrix. A nomenclature is given in the legends to Pl. 1, fig. 2.

#### (b) *Summary of test experiments*

(1) Unerupted or erupted molars or incisors, cultured as a whole (Pl. 1, figs. 1, 4 and 5; Pl. 2, figs. 13, 15 and 18). It was found that the rate of growth varied with the age *in vivo* of the explant. The enamel organ still containing the four layers described above gave rise to epithelial sheets or cords (Pl. 2, figs. 13, 15 and 18), whereas the reduced enamel organ did not give rise to epithelial outgrowth. In all cases, except one, the enamel organ of incisors gave rise to epithelial outgrowth on the labial side and on the proximal end only. (In the one exceptional case, epithelial outgrowth occurred on the lingual side as well.)

(2) Extirpated pulps from unerupted molars gave rise to outgrowth of fibroblasts and wandering cells.

(3) Enamel organs of unerupted molars were not successfully cultured, probably because of damage during dissection.

(4) Extirpated pulps and growing ends of incisors (Pl. 1, figs. 6, 7). When these two tissues were explanted just after birth, abundant outgrowth was observed from both (Pl. 1, fig. 6), but as the age *in vivo* of the explant increased, this outgrowth soon became restricted to the growing end (Pl. 1, fig. 7). Only a few cells migrated out from the pulp. In all instances the growing end is surrounded by a large area of outgrowth. Further analysis of the outgrowth from incisal cultures is given in the following paragraph.

(5) Extirpated pulp of incisors gave rise to fibroblastic outgrowth and some wandering cells. This outgrowth originated from the most proximal end of the explant.

(6) Explants of dissected and isolated growing end of incisors, of all ages *in vivo*, gave rise to outgrowth. These consist of wandering cells, fibroblasts, 'flattened fibroblasts' (see para. (c)) and epithelial cells.

(7) Dissected enamel organ and periodontal membrane of incisors gave rise to epithelial outgrowth on their proximal parts only, i.e. where the enamel organ forms

the enamel matrix *in vivo*. The distal end, where the maturation was in progress, did not give rise to epithelial outgrowth. The periodontal membrane gave rise to fibroblast outgrowth from all parts of the explant.

(8) Cultures of the hard tissue residuum will be discussed in the next paragraph, with some other aspects of the outgrowth from incisor cultures.

(c) *Outgrowth of odontoblasts from incisor cultures*

It was found that not only the rate but also the pattern of the outgrowth varied with increasing age *in vivo* in a regular and characteristic way. Special attention was therefore paid to cultures of whole, undamaged pulp and growing-end cultures of incisors, in which it was possible to localize the sources of outgrowing cells.

As was expected, wandering cells, fibroblasts and epithelial cells migrated out from the growing end. These cells are regularly distributed around the growing end, as they are found, for example, around an explanted embryonic kidney; but one particular cell type repeatedly occurs in two positions: on the labial and lingual junctions of the pulp with the growing end (Pl. 1, fig. 7 *C* and *D*). These cells build up an epithelium-like mosaic when just leaving the explant, but towards the margin of the outgrowth they become more and more fibroblast-like and isolated. They occupy a larger area on the labial than on the lingual side. Sometimes they also appear on the pulp itself.

In order to localize their origin, pulp deprived of the growing end, and the growing end itself, were explanted separately. It was found that the pulp on its labial and lingual surface gave rise to a not very abundant fibroblastic outgrowth, whereas the cut proximal end was the source of a rich outgrowth, consisting of fibroblasts and some of the flattened cells of quasi-epithelial habit mentioned above. The orientation of a growing-end culture was not possible; outgrowth from it consists of wandering cells, ordinary fibroblasts and flattened cells.

A second way of localizing the source of these flattened cells was to culture the 'hard tissue residuum' (Pl. 2, fig. 8). This residuum consisted of dentine, enamel, predentine and enamel matrix, after the 'soft dental tissues' (pulp, enamel organ and growing end) had been dissected away. Because it was never certain whether or not this dissection was complete, there was always a chance that some cells of the soft tissues remained attached to the residuum. When this residuum was cultured, the same flattened cells appeared on the proximal end of the explant, resembling epithelial tissue near the explant, but becoming more fibroblast-like towards the periphery.

There is a possibility that these flattened cells represent the outgrowth of an epithelium from the enamel organ, which later becomes surrounded or overgrown by fibroblasts. To compare outgrowth from the enamel organ with these mosaic sheets or flattened cells, see Pl. 2, fig. 9, where epithelial cells migrated out from the residuum, or the epithelial sheets of Pl. 2, figs. 13, 15 and 18. It is easy to see that these two sheets differ: the sheet in fig. 8 is built up of cells of similar shape, attached to each other less closely than those in fig. 13, and changing their shape to fibroblast-like cells at the edges. On the other hand, the sheet in fig. 13 is typically epithelial, the cells being closely attached to one another and not 'transforming' into fibroblasts at the edges.



(d) *The characteristics of epithelial outgrowth*

As shown in Pl. 2, figs. 9, 13, 15 and 18, the main characteristics are: growth in sheets or cords; the nuclei (unlike those of odontoblasts) of variable shape. The cells have a tendency to become isolated at the edges of the sheet.

(e) *Periodontal membrane*

Very abundant, typically radiating fibroblast outgrowth was obtained from the periodontal membrane.

B. *Histological changes in vitro*

Changes consequent upon dedifferentiation in hanging-drop cultures will be the first to be described.

(a) *Dental papilla and pulp*

It was found that the dental papilla, when explanted *in situ*, i.e. in a culture of the whole tooth germ, did not change histologically (Pl. 1, fig. 1; Pl. 2, fig. 16), whereas the pulp, also *in situ*, showed a regular transformation. The pulp became divisible into three zones (Pl. 1, fig. 4). The normal structure in the crown did not change. Next to this region a comparatively cell-free area developed *in vitro*. At the apical foramen, however, the entire pulp became reorganized; even odontoblasts took part in this change. The fibroblasts inside the pulp, which have no particular orientation *in vivo*, became orientated *in vitro* in such a way that their long axes were perpendicular to the dentine. Consequently, they built up a bridge across the apical foramen. The appearance in sections very strongly suggests the idea that the odontoblasts along the predentine and the fibroblasts from the cell-free zone had migrated towards the apex.

A few extirpated incisor pulp cultures were also sectioned (Pl. 2, figs. 11 and 12). The tip of the pulp (Pl. 2, fig. 12) remained almost unchanged and the odontoblasts remained alive, although the Tomes's fibres were damaged. Towards the growing end and inside it (Pl. 2, fig. 11) the pulp cells, including the odontoblasts, showed a tendency to migrate into the medium. In the tip the number of fibroblasts increased underneath the odontoblastic layer, and did not grow out into the medium. The odontoblasts remained in their original arrangement and did not grow out either.

The dentine formed prior to explantation showed no change: no 'calciotraumatic line' was found. The structure of the dentine forming in the developing root changed into a bone-like structure in the following manner (Pl. 2, fig. 14). Between the odontoblasts fibrous trabeculae appeared, enclosing one or more odontoblasts, separating them from the pulp itself. At the same time the surface of the predentine became 'scalloped'. No secretion of matrix around the odontoblasts was observed.

The predentine forming in the growing end of an incisor explant formed irregular loops, and when the predentine was injured during explantation, fibrous repair tissue formed *in vitro* (Pl. 2, fig. 10).

(b) *Epithelial tissue*

The epithelial tissue of the teeth also showed 'adaptive changes' *in vitro*. The 'young' enamel organ, with its four layers, gave rise in parts to a stratified squamous epithelium (Pl. 2, fig. 16), which did not keratinize, whereas the oral epithelium

formed a keratinized cyst over the tooth germ (see also Pl. 1, fig. 1). This difference occurred in every specimen. When the enamel organ was reduced in size *in vivo* (see above), the source of epithelial migration was usually located where the epithelial sheath of Hertwig joins the enamel organ.

In two cultures (in exactly the same manner in both) the outer enamel epithelium of Hertwig's sheath showed an interesting transformation *in vitro* (Pl. 2, fig. 17; Pl. 3, fig. 21). The epithelium was in immediate contact with the culture medium. The cells became columnar, their nuclei came to lie away from the medium, being thus 'polarized' much as secreting cells are polarized towards their discharging pole. No secretion was, however, observed.

## SECTION II. ORGAN CULTURES IN FLUID MEDIA

The purpose of these cultures was to observe day by day the transformations or degenerative changes of undissected mouse incisors in media with or without serum. The behaviour of the growing end, the enamel organ, the pulp and the alveolar bone will be described separately.

(1) The *growing end* of the incisor underwent a slower transformation *in vitro* than the pulp or the enamel organ. The epithelial loops degenerated in a medium without serum (Pl. 3, fig. 25), but when serum was used they survived, showing a slight stratified squamous transformation (Pl. 3, fig. 23). This transformation may be connected with proliferation into the pulp cavity of short epithelial cords using the fibrous network of the pulp as a natural substratum. In cultures with serum (and especially in high  $O_2$  concentration) mesenchymal cells also invaded the pulp; these originated from the growing end or from the periodontal tissue (Pl. 3, figs. 24 and 27). When no serum was added to the medium, the mesenchymal tissue in the growing end did not invade the pulp but merely survived, without change, for a longer period than the epithelial loops.

(2) The *enamel organ* did not undergo transformation in a medium without serum; it merely degenerated (Pl. 3, fig. 25). No sign of matrix formation was observed. When serum was used in the medium, the apical, middle and incisal thirds of the enamel organ responded differently to explantation. These changes were best observed when incisors were cultured in a high  $O_2$  gas phase (Pl. 3, figs. 20, 24 and 27).

In the apical third (Pl. 3, figs. 24 and 27), where no enamel matrix is found, the enamel organ became folded soon after explantation and later, with increasing age *in vitro*, the ameloblasts degenerated and the whole enamel organ became squamous. Large, cyst-like formations are also observed in this area, which never keratinize. Generally, the apical third is the most sensitive part of the enamel organ and the earliest to degenerate.

The middle third (over the enamel matrix) transformed in the following way *in vitro* (Pl. 3, fig. 20): first, the ameloblasts became more widely spaced than they were *in vivo*: then they were pulled away from the matrix. Finally Tome's processes disappeared and the ameloblasts shortened. When this change is complete, a typical stratified squamous epithelium covers the area (Pl. 3, fig. 22). This change may be further connected with the formation of globules inside the enamel organ (Pl. 3, figs. 20 and 23). These globules stain like the enamel matrix. The structure of the outermost layer of the enamel matrix shows hypoplastic changes. In some

specimens cells from the periodontal membrane also invaded this hypoplastic area (Pl. 2, fig. 19), and penetrated as far as the enamel matrix, where they are found to occupy small or large depressions on the surface of the matrix.

In both series of cultures, whether with or without serum, the incisal third of the enamel organ (over enamel, with short ameloblasts) degenerated and no squamous transformation is found.

(3) The *pulp* showed a remarkable capacity for survival. Whereas the enamel organ and the periodontal tissue degenerated in a medium without serum, the pulp survived, although slightly altered in structure (Pl. 3, fig. 25). The blood vessels, naturally, became obliterated, the ground substance became fibrous and the stellate pulp cells changed to round or spindle-shaped cells. Finally the number of cells decreased in the centre of the pulp. Where the pulp remained healthy, the odontoblasts survived without changing their shape.

No 'calciotraumatic line' (see above) is found in the dentine, but the predentine nearest the growing end forms irregular loops (Pl. 3, figs. 23, 24 and 27). No apparent major disturbance in calcification is observed, so far as it is possible to judge this by comparing the number of calcosphaerites and the width of the predentine with the corresponding observations on the controls. Sometimes also the dentine is invaded on the lingual side by cells from the periodontal membrane.

(4) The *periodontal tissue* degenerated in a medium without serum but survived when serum was added. As was mentioned above, this surviving tissue sometimes invaded the underlying enamel organ (Pl. 2, fig. 19).

In addition, study of experimental injury was also carried out *in vitro*. On two occasions fractured incisors were cultured (Pl. 3, fig. 26). The pulp nearest to the injury died, but it remained healthy further away from the injured part. Deposition of not very clearly differentiated fibrous tissue was also found around a broken and intruded piece of dentine.

## DISCUSSION

### (a) 'Flattened cells' as odontoblasts

The evidence gained from hanging-drop cultures strongly suggests that the flattened cells which form sheets proximally, but a fibroblast-like reticulum peripherally are outgrowing odontoblasts. The crucial question of their capacity to form calcified tissue *in vitro* is still open, but indirect evidence seems to prove their identity with odontoblasts. It has been shown by several authors that, under certain conditions, odontoblasts may change shape *in vitro*. Wolbach & Howe (1933) state that in vitamin A deficiency the odontoblasts atrophy and lose their polarization, becoming spindle-shaped cells. Schour, Brodie & King (1934) state that in certain pituitary disturbances the odontoblasts in some areas fuse into a syncytium, giving the appearance of a stratified squamous epithelium. The present evidence of culturing pulps *in situ* in hanging-drop cultures shows that in the area of root formation, or near the growing end, the odontoblasts are transformed into spindle-shaped cells and lose their pattern of orientation.

Although no proof of function was gained, in one particular point the outgrowing odontoblasts resembled their normal counterparts *in vivo*: they adhered to one another along their cell membranes.



The third point in favour of their identity is that these flattened cells always grow out from the same part of the incisor: from the junction of the growing end and the pulp, or from the apical end of the hard tissue residuum. In the latter case some odontoblasts may have been left adhering to the predentine after the pulp was extirpated and these cells then migrated out into the medium. Manley & Marsland (1952), describing changes in pulps after fracture, also mention that 'proliferation and migration of the older cell lining the walls of the pulp and root canal cannot be ruled out entirely'. In the present experiments the culture of the hard tissue residuum may suggest that this migration is possible. The marginal area of the growing end of a pulp of an incisor can also be regarded as the locus of already differentiated odontoblasts which have not yet formed large masses of dentine. The marginal area corresponds to the apical end of the hard tissue residuum.

#### (b) *Epithelia*

The epithelial sheets showed a wide range in nuclear shape *in vitro*. This is in accordance with findings *in vivo*: Brügger (1949) pointed out the same fact when measuring the nuclear shape in enamel organ.

#### (c) *Internal changes; the pulp*

Generally speaking, the pulp is the most resistant tissue *in vitro*, preserving its normal structure. It is remarkable that even in a serum-free culture medium, it survived as long as 3 weeks, although other dental tissues degenerated much earlier. The pulp is also very resistant *in vivo*; a normal pulp often has been found in fractured teeth long after injury. The pulp also preserves its normal structure in transplants, as it was observed by Huggins, McCarroll & Dahlberg (1934) and by Hahn (1941).

The differentiation into three zones found in hanging-drop cultures of dental pulps of molars is somewhat similar to changes in vitamin D deficiency described by Blackberg & Berke (1932) in the following terms: '... There is some degeneration of the stellate cells in the upper portion of the pulp, while those nearer the apices appear quite normal. The cells near the areas of fibrosis... assume the characteristics of fibroblasts.' The fibroblastic barrier built across the apical foramen in cultures of whole teeth may be regarded as equivalent to a repair process *in vivo* (cf. Szabó, 1928; Manley & Marsland, 1952).

The changes of the pulp during culture in fluid media are in some ways comparable with changes associated with age and magnesium deficiency *in vivo*. Bevelander (1941) stated that mature teeth have a more extensive fibrous system than immature teeth; it is also known that in atrophy and fibrosis the cellular elements of the pulp disappear, being replaced by collagenous fibres. Similar observations were made by Orban (1949) in his study of ageing, and by Becks & Furuta (1942), who observed that the stellate cells of the pulp became rounded in magnesium deficiency.

Odontoblasts may survive as long as the other cells in the pulp. Their degeneration begins at the growing end, *in vitro*, as it does in some cases *in vivo* (Wolbach & Howe, 1933; Irving, 1943; Banks, Bhaskar & Weinmann, 1951; etc.).

The absence of a 'calciotraumatic line' in these cultures may suggest that calcification stopped *in vitro*. There is, however, some evidence that it continued. Predentine

formation (loop formation) certainly continued *in vitro*. If this is so, and calcification stops, a significant change in the proportion of the width of dentine and predentine should have arisen. This was not the case. Moreover, calcification in teeth and bone is known to continue *in vitro* (Glasstone, 1936, 1938; Nuckolls, 1941 *a* and *b*; Fell, 1932). Lastly it may be pointed out that the odontoblasts over a large area of the pulp did not show degenerative changes *in vitro*.

The changes shown in Pl. 2, fig. 14, are similar to the 'calcific repair tissue' of Fish (1939), although at such an early stage as that, it is not possible to say whether the process would result in the formation of repair tissue or scar tissue.

It was found that cellular invasion of the pulp *in vitro* originates from the growing end of the incisor. Wolbach & Howe (1933) and Aisenberg (1943) found the same phenomenon under certain experimental circumstances *in vivo*, and it is well known that pulpless teeth may be invaded through the apical foramen (e.g. Lefkowitz, Bodecker & Shapiro, 1944). Deposition of cementum was not observed *in vitro* after cellular invasion, probably because of the short duration of the experiments.

It may be mentioned that in the few experimental injuries *in vitro* the pulp behaved as it does *in vivo*: it dies only in immediate neighbourhood of the injury.

#### (d) *The enamel organ*

The enamel organ became in parts transformed into stratified squamous epithelium. It is significant that, unlike epidermal explants (cf. Hanson, 1950), the enamel organ did not form keratinized cysts in hanging-drop cultures. This difference is also emphasized by Huggins, McCarroll & Dahlberg (1934), who did not observe keratinized cysts deriving from enamel organ in transplants, whereas the oral epithelium, like *in vitro*, readily formed such cysts.

In fluid medium cultures the enamel organ of incisors reacted in its middle third, as Weinmann (1943) found it after injection of strontium chloride, i.e. the first sign of transformation appeared here; whereas the apical area folded and formed (not keratinized) cysts, as Schour, Chandler & Tweedy (1937), Weinmann & Schour (1945) had found under various experimental conditions.

The sharp differences between the incisal and middle thirds of the enamel organ in their capacities for transformation *in vitro* suggests that there is a basic physiological difference between the short (incisal third) and the long (middle third) ameloblasts. This result supports the opinion that histologically enamel maturation must be clearly distinguished from matrix formation as functions of the enamel organ, and that the cellular changes at the end of matrix formation represent the start of maturation (Marsland, 1952). This distinction is manifested *in vitro* by different reactions to explantation: the enamel organ over the matrix undergoes transformation, while over the enamel it merely degenerates.

The invasion of the enamel organ by connective tissue cells (Pl. 2, fig. 12) is a consequence of the breaking down of the enamel organ *in vitro*. A similar observation was made by Lefkowitz *et al.* (1944) *in vivo*, who also mention resorption of the enamel matrix in the invaded area. In our experiments the hollow surface of the matrix, with cells occupying these hollows, also strongly suggests resorption.

Lastly, it may be mentioned that the observations described here provide a new illustration of the well-known theorem that rate of growth and degree of differentia-

tion vary inversely. Under the same experimental conditions, and even in the same culture vessel, the pulp and the incisal and medial thirds of the enamel organ give rise to little outgrowth, while the growing end and the apical third of the enamel organ are surrounded by dense outgrowth. Correspondingly, the odontoblastic layer of the pulp and the incisal and medial thirds of the enamel organs may perhaps be regarded as the most highly differentiated dental tissues.

#### SUMMARY

1. Molar germs and unerupted or erupted incisors, both undissected and dissected into several parts—pulp, enamel organ, growing end and the remainder of the hard dental tissues—were grown in hanging-drop and fluid media (rocker flask) cultures. Streptomycin was used as an antibiotic.

2. Evidence is given that odontoblasts survived and grew *in vitro* and their mode of outgrowth is described. Odontoblasts, though individually spindle-shaped, tended to coalesce into sheets.

3. The structure of outgrowing epithelial sheets is also described. There is found to be a great variety of nuclear shape and a tendency at the culture periphery towards isolation of the outgrowing cells.

4. Histological changes in the explants were examined. The pulp is the most resistant of dental tissues to the changed environment *in vitro*, and shows changes comparable with repair and ageing *in vivo*.

5. The enamel organ of incisors in fluid media became transformed into stratified squamous epithelium in its apical and middle thirds, a change associated with the formation of 'hypoplastic' enamel matrix. The incisal third, however, is incapable of dedifferentiation *in vitro*.

6. The oral epithelium formed a keratinized cyst, whereas the enamel organ did not keratinize.

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## EXPLANATION OF PLATES

Unless the contrary is stated, the specimens were stained with Harris's haematoxylin and counterstained with eosin. All specimens, except fig. 11, are teeth of mice. Figs. 1-18 and 21 are hanging-drop cultures, figs. 19 and 22-27 fluid medium cultures.

## PLATE 1

- Fig. 1. Section through a first molar germ, 17th day *in utero*, 5 days *in vitro*. *A*, keratinized cyst, derived from the oral epithelium; *B*, enamel organ with four layers, *C*, dental papilla.  $\times 120$ .
- Fig. 2. Tangential section of an erupted mouse incisor in socket, apical half. *A*, loop of the enamel organ in the growing end; *B*, apical or proximal end of labial dentine. The outgrowth illustrated in Pl. 2, figs. 8, 9, originated from this area; *C*, enamel matrix on the labial, convex aspect; *D*, pulp; *E*, odontoblastic layer; *F*, dentine. The light stained line between dentine and odontoblastic layer is predentine. *F* points to the border between the dentine and the predentine in the distal or incisal end of the tooth. Note the absence of enamel on the lingual, concave aspect.  $\times 20$ .
- Fig. 3. Section through a second molar germ, 34th day after fertilization: control for fig. 4. Compare the pulp with that in fig. 4. The enamel organ still covers the enamel. Arrows point to the epithelial sheet of Hertwig in the developing root.  $\times 55$ .
- Fig. 4. Section of a second molar germ from the same animal as in fig. 3, 3 days *in vitro*. Weigert-van Gieson. *A*, unaltered zone in pulp; *B*, relatively cell-free zone; *C*, fibrous tissue barrier in the forming root.  $\times 90$ .
- Fig. 5. Living culture of a whole unerupted incisor, 5 days *in vitro*. *A*, growing end; *B*, tip of developing incisor.  $\times 23$ .
- Fig. 6. Whole mount of a culture of the growing end and pulp of an incisor. Newborn mouse, 4 days *in vitro*. Note the abundant outgrowth from the whole specimen.  $\times 27$ .
- Fig. 7. Whole mount of the 'growing end' component of a combined pulp and growing end culture. Erupted incisor, 4 days *in vitro*. *A*, growing end; *B*, beginning of pulp; *C* and *D*, outgrowing odontoblasts on the labial (*C*) and on the lingual (*D*) side.  $\times 35$ .

## PLATE 2

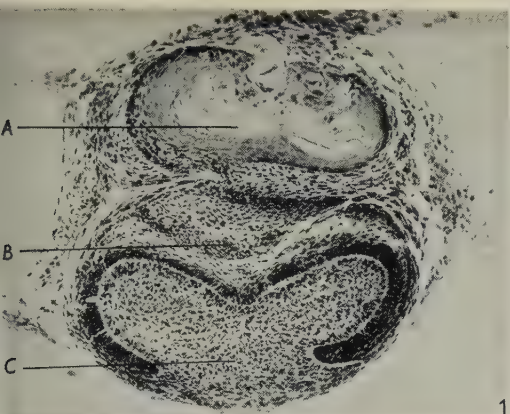
- Fig. 8. Whole mount of a culture of the 'hard tissue residuum'. Unerupted incisor, 4 days *in vitro*. Outgrowing odontoblasts forming a sheet near the original explant and becoming fibroblast-like and isolated at the margin of the sheet.  $\times 90$ .
- Fig. 9. Another whole mount of the hard tissue residuum. Unerupted incisor, 5 days *in vitro*. Outgrowth of epithelial sheet, different from that of fig. 8.  $\times 90$ .
- Fig. 10. Section through a first molar from a newborn mouse, 3 days *in vitro*. Deposition of scar tissue in the injured part of the dentine (arrow). Note also the three zone arrangement of the pulp.  $\times 150$ .
- Fig. 11. Section through the culture of the marginal area between growing end and pulp of a rat incisor. Labial side, 2 months old rat, 4 days *in vitro*. Outgrowth of young odontoblasts and fibroblasts from the explant. The outgrowth is very similar to that of odontoblasts in fig. 8, and differs from that of fig. 9 (epithelial outgrowth).  $\times 180$ .
- Fig. 12. Section through a hanging-drop culture of an extirpated incisor pulp. Erupted incisor, 3 days *in vitro*. The area shown is near the incisal tip. No outgrowth. Accumulation of fibroblasts underneath the odontoblastic layer.  $\times 150$ .
- Fig. 13. Whole mount of a culture of a second molar germ, 19th day *in utero*, 4 days *in vitro*. Large outgrowth of epithelium.  $\times 35$ .
- Fig. 14. Section through a culture of a first molar from a newborn mouse after 1 week *in vitro*. Deposition of calcific scar tissue on dentine formed prior to explantation (arrow).  $\times 150$ .
- Fig. 15. Whole mount of a culture of a third molar, 31st day after fertilization, 3 days *in vitro*. *A-B*; fibroblasts and epithelial sheet in the outgrowth around the explant.  $\times 45$ .
- Fig. 16. Section through a culture of a second molar germ, 20th day *in utero*, 5 days *in vitro*. *A*, keratinized cyst, full of cellular debris, formed between the oral epithelium and the tooth germ; *B*, outer enamel epithelium and stellate reticulum transformed into squamous epithelium.  $\times 100$ .

- Fig. 17. Section through a first lower molar, 20th day after fertilization, 4 days *in vitro*. Mallory connective tissue stain. Example of the columnar epithelium, formed *in vitro* from the epithelial sheet of Hertwig and polarized towards the medium.  $\times 150$ .
- Fig. 18. High-power photograph of a part of fig. 15. Epithelial cells and fibroblasts in the out-growth. Note the great variety of nuclear shapes.  $\times 185$ .
- Fig. 19. Section through the enamel organ of an erupted incisor, cultured in medium with serum for 11 days. *A*, enamel matrix formed before explantation; *B* and *C*, invasion and partial resorption of matrix  $\times 300$ .

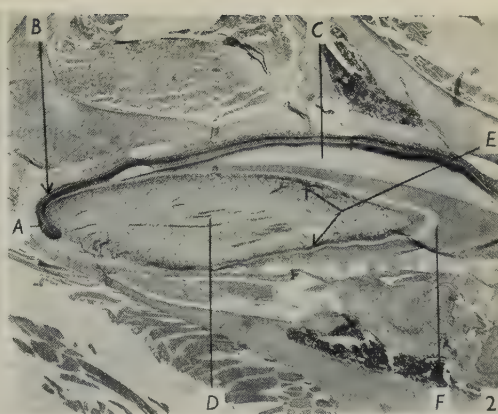
## PLATE 3

- Fig. 20. Section through the area *A* of fig. 27. Weigert-van Gieson. *A*, predentine; *B*, dentine; *C*, enamel matrix, becoming hypoplastic towards *D*, the distorted ameloblastic layer, which has partly lost its columnar shape; *E*, enamel-matrix like globules formed inside the enamel organ. Note the large cyst inside the enamel organ.  $\times 295$ .
- Fig. 21. Section through a first lower molar, 5th day after birth, 5 days *in vitro*. Arrow points to the columnar epithelium at the wide cervical opening, formed from epithelial sheath of Hertwig.  $\times 50$ .
- Fig. 22. Section through the enamel organ of an erupted incisor. Culture in medium with serum for 14 days. Stratified squamous epithelium over the enamel matrix, formed from the enamel organ *in vitro*.  $\times 150$ .
- Fig. 23. Longitudinal section through an erupted incisor with its socket, cultured for 8 days in medium with serum. *A*, labial; *B*, lingual loops of the enamel organ in the otherwise degenerate growing end; *C*, ectopic enamel matrix formed in the surviving middle part of the enamel organ. Pulp healthy towards the tip (on the right).  $\times 27$ .
- Fig. 24. Area *B* of fig. 27. *A*, transformed enamel organ; *B*, loops of newly formed dentine in the growing end, where the fibroblasts (and some of the dedifferentiated odontoblasts) begin to invade the pulp (towards the left).  $\times 230$ .
- Fig. 25. Longitudinal section through an erupted incisor in socket, cultured for 6 days without serum. Same animal as in fig. 23. The enamel organ has degenerated, but the pulp is healthy towards the incisal tip (left).  $\times 27$ .
- Fig. 26. Longitudinal section through an erupted incisor, cultured in fluid medium without serum for 10 days. Experimental injury. A hole was made through the enamel matrix (*A*) and dentine (*B*), and a piece of dentine (*C*) was inserted inside the pulp. Area of necrosis under the hole, fibrocyte reaction around the inserted piece of dentine.  $\times 75$ .
- Fig. 27. Tangential section through an erupted incisor in socket. From a member of the same litter as in figs. 23, 25. Cultured in serum under high  $O_2$  tension. Area *A* (cyst and ectopic enamel matrix formation), inside the enamel organ, is shown in fig. 20. *B*, the area near the growing end, is shown in fig. 24. Note the proliferation from the alveolus at *A*.  $\times 27$ .

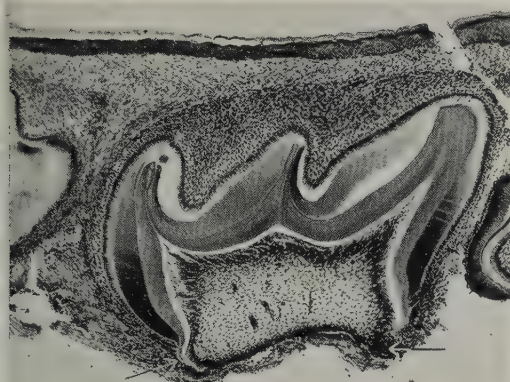




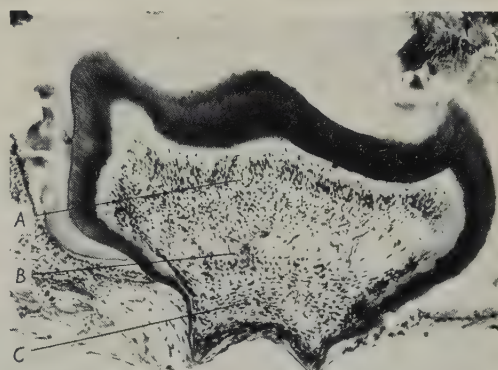
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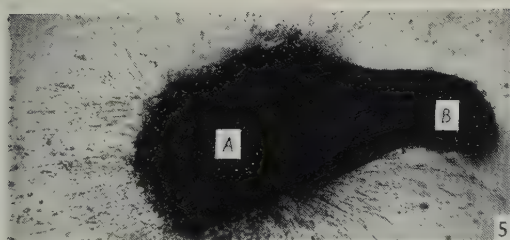
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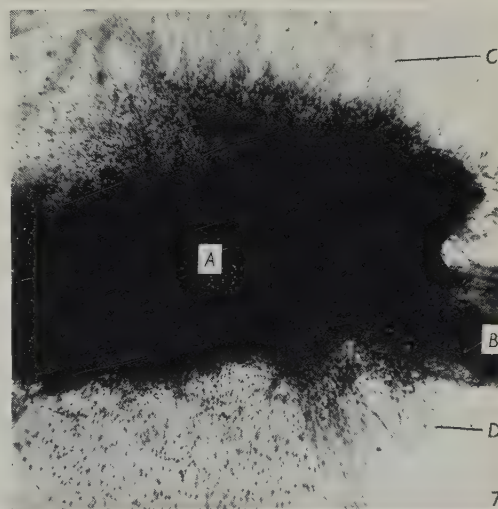
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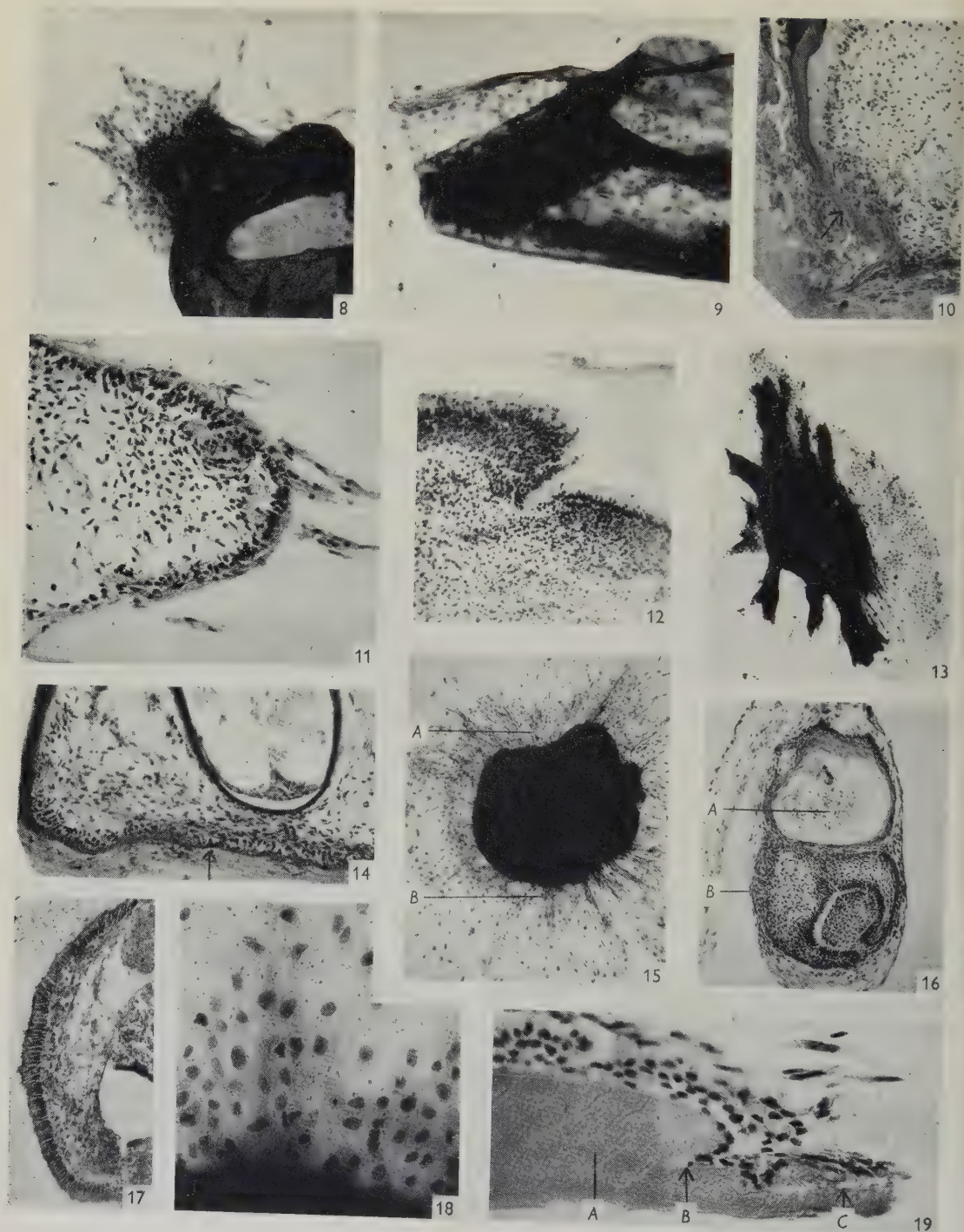
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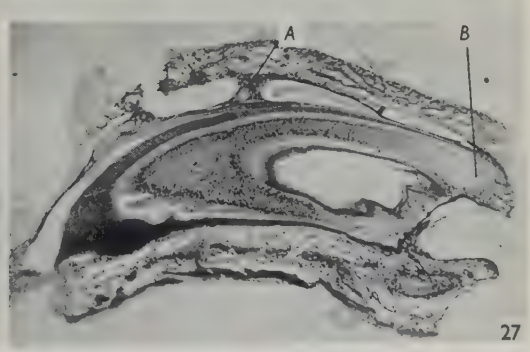
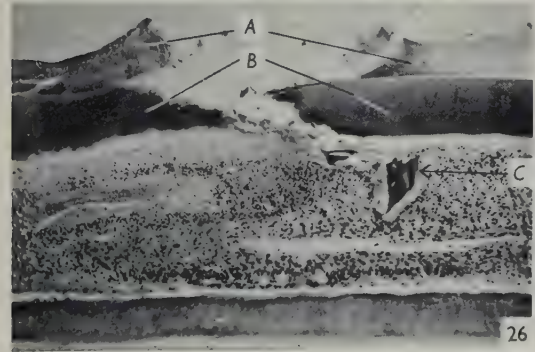
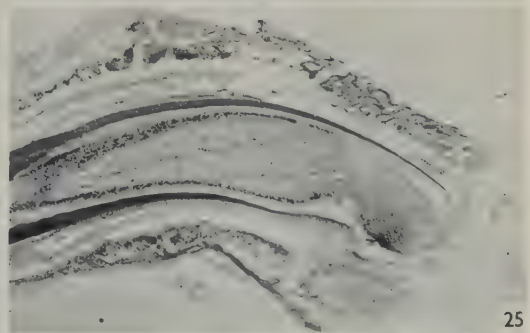
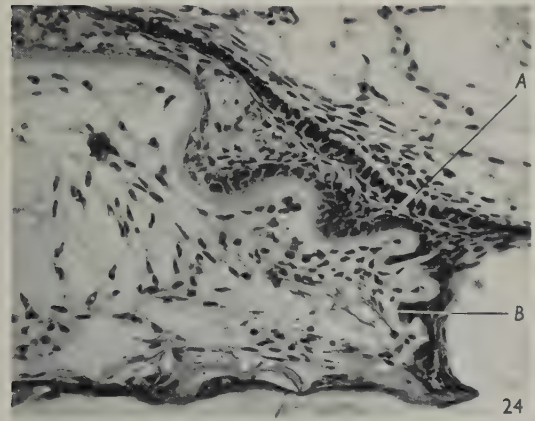
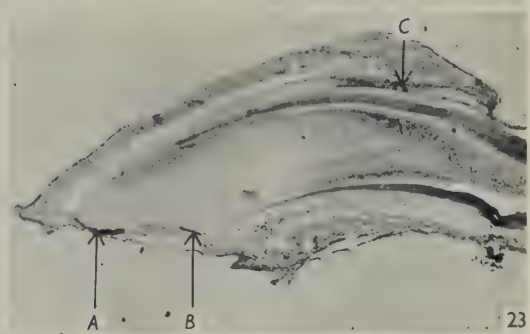
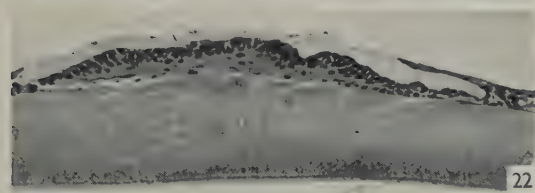
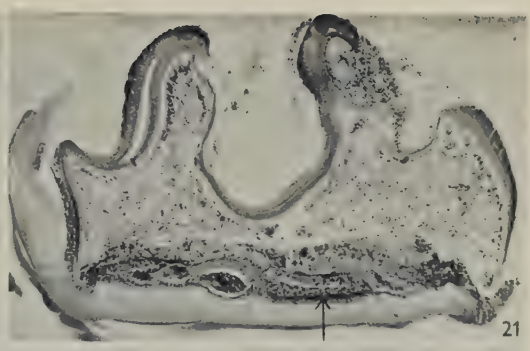
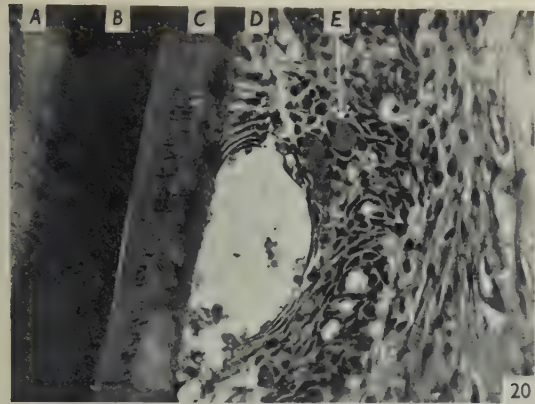
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# THE EPIDERMAL MELANOCYTES OF MICE

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## INTRODUCTION

It is an accepted fact that pigmentary dendritic cells (epidermal melanocytes) occur in the matrices of pigmented hairs in mice, and in the basal layer of the surface epidermis in regions of the body where hairs are sparse, e.g. the tail, the sole of the foot, and the ears (Steiner-Wourlish, 1925; Rawles, 1947). No report has been made, however, of the occurrence of dendritic cells in the unpigmented epidermis of the densely haired skin of the trunk. In recessively spotted black-and-white guinea-pigs and Friesian cattle, in which pigmented hairs issue from pigmented skin and white hairs from pigment-free skin, it has been demonstrated that dendritic cells are a constant ingredient of the epidermis irrespective of whether it is pigmented or not (Billingham, 1948); there is, in fact, a complete network of dendritic cells throughout the epidermis. The purpose of the present investigation has been to make a thorough survey of the distribution of dendritic cells in self-coloured agouti mice, and in 'recessively spotted' black-and-white mice.

In this paper, epidermal dendritic cells which possess the capacity for elaborating melanin will be called *pigmentary* melanocytes. Those pigmentary dendritic cells which actually contain visible melanin are referred to as pigmented or 'active' melanocytes. Dendritic cells which do not contain melanin and which cannot be induced to form it, will be called non-pigmentary melanocytes. This terminology follows Billingham (1948) except in the substitution of 'melanocyte' for 'dendritic cell' as a result of an informal agreement among workers on pigmentation arrived at on the initiative of Drs Fitzpatrick, Lerner, and Pincus. 'Dendritic cell' will continue to be used as a non-committal general term whenever the particular classification is under discussion or in doubt.

## MATERIAL AND HISTOLOGICAL METHODS

The major part of this investigation has been carried out on a stock of hybrid agouti mice; the spotted animals required for later work came from a partially inbred spotted black-and-white strain.

When skin was required for histological examination the mice were killed with coal gas and the area to be studied at once clipped and shaved. As a general rule fragments of skin, approximately 5 by 5 mm., were so excised as to include the full thickness of the epidermis with as little of the corium as possible. Since the epithelia of the tongue and the vagina are stratified and comparable in their histological structure with that of the integument, they have been included in this investigation, although neither of them is of ectodermal origin.

All material was examined as stained transverse paraffin sections after fixation in formol-mercuric chloride, and as whole mounts of 'pure' epidermis. Sections

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were cut 8-10  $\mu$  thick and lightly stained with Ehrlich's haematoxylin and eosin, or Mayer's carmalum. Sheets of pure epidermis completely free from any dermal elements were prepared by the tryptic digestion method fully described by Billingham & Medawar (1951). In order to facilitate manipulation during staining operations the sheets of epidermis were prepared in such a way as to leave them 'glued' out flat on small fragments of glass cover-slip, with their basal layer surface outermost, after removal of the corium (see Billingham & Medawar, 1953). The operation for preparing these epidermal sheets will be referred to hereafter as 'skin splitting'. They were then either stained supra-vitally\* or fixed and stained by ordinary methods. Some difficulty was experienced in obtaining sheets of pure epidermis from the trunk skin of mice, partly because of the abundance of hair follicles, and partly because the corium of the skin of the trunk was found to be relatively resistant to the action of the trypsin solution; it was therefore obligatory to use the thinnest possible shavings. These were cut by pulling the rather mobile skin as tautly as possible over the forefinger, and making very shallow horizontal strokes with a sharp straight-edged scalpel (e.g. with a no. 11 blade). Such shavings required only 10 min. tryptic digestion to achieve the separation of the corium.

#### *Staining methods*

##### *Silver impregnation*

Even the finest melanin particles can be caused to blacken almost specifically when, after careful washing, formol-fixed tissue is incubated for 1-3 hr. at 37° C. in a 0.01% aqueous solution of silver nitrate made very slightly alkaline by the addition to 100 ml. of one drop of concentrated (sp.gr. 0.88) ammonia solution. No reduction is necessary: unreduced silver is removed with sodium thiosulphate solution. Both paraffin sections and sheets of pure epidermis were impregnated in this manner, and the distribution of melanin throughout the epidermis was thus clearly revealed. This technique also enabled a comparison to be made between the melanin content of the dendritic cells and of the neighbouring Malpighian cells.

##### *The 'Dopa' reaction*

When a piece of fresh or briefly formol-fixed tissue is incubated in a solution of 3:4: dihydroxyphenylalanine ('Dopa') at pH 7.4, certain cells are able to bring about its intracellular oxidation to Dopa-melanin, becoming intensely blackened in the process. Such cells are said to be 'Dopa-positive'. In the epidermis this reaction is specific to those melanocytes that normally display pigmentary activity. Throughout this investigation, the standard procedure of Laidlaw & Blackberg (1932) was used, and for carrying out the reaction both freshly prepared sheets of pure epidermis and frozen sections were employed. Where paraffin sections were found to be necessary, as when studying the distribution of melanocytes in hair follicles, specimens comprising the full thickness of the skin were subjected to Dopa treatment after a brief preliminary period of formol fixation. They were then given an additional period in the fixative and sectioned by ordinary methods.

\* Experiments have shown that sheets of pure epidermis prepared in this way remain alive and can be used as grafts (Billingham & Medawar, 1950, 1951; Billingham & Reynolds, 1952).



*Supra-vital staining with brilliant cresyl blue*

This technique has been described by Billingham & Medawar (1953). The stain was made up immediately before use by adding 8.5 ml. of Ringer-bicarbonate to 1.5 ml. of stock solution of 1 : 1000 brilliant cresyl blue in distilled water. A few ml. of this solution were placed in open shallow watch-glasses, and to these were added the cover-slips with freshly prepared sheets of pure epidermis mounted upon them. Staining was carried out at room temperature, and the preparations were examined microscopically every 15 min., the cover-slip being inverted to present the basal layer of the sheet of epithelium uppermost. After about half an hour, specifically stained branching elements could be distinguished. The preparations reached their optimum condition for study after 1–3 hr. A great disadvantage of this technique is the rapid deterioration of the preparations after the optimum condition has been attained, and no satisfactory method has yet been devised for making them permanent.

*Gold impregnation*

The method adopted was essentially that used by Gairns (1930) for demonstrating nerve endings in muscle; Billingham (1948) has successfully employed a modification of it for 'staining' dendritic cells. Briefly, it consists of soaking freshly excised skin shavings in a 1 % gold chloride solution for 10 min. after a short period of fixation in a 1 : 3 mixture of formic acid and freshly expressed lemon juice. After several hours' treatment with a 25 % solution of formic acid, the tissue is cleared in glycerine. At this stage the epidermis is easily separated from the swollen corium and mounted, basal-layer uppermost in pure glycerol.

Further experimental techniques will be described in the text.

## OBSERVATIONS

(1) *Agouti mice*

*General.* The supra-vital staining of sheets of 'split skin' with brilliant cresyl blue reveals the presence of a complete system of dendritic cells in the basal layer of the epidermis throughout the body. (The peculiarities of each region will be dealt with below.) Overt pigmentation is, however, confined to relatively hairless and exposed regions such as the tail, the ear tips, and areas on the sole of the foot. Correspondingly, the melanocytes of these regions are in an active pigmentary state, being Dopa-positive and loaded with melanin granules. In some regions, e.g. the ear, there is a gradient of pigmentary activity among the melanocytes; at the ear tip, they are strongly Dopa-positive and heavily loaded with melanin. Passing proximally, the Dopa reaction becomes confined to scattered groups of melanin-containing cells until, at the base of the ear, the Dopa reaction and melanin content are lost and the melanocytes can be revealed only by supra-vital staining with brilliant cresyl blue. Evidence will be given later that in both body skin and in ear skin, these unpigmented melanocytes are *potentially* pigmentary in function: they should therefore be classified with the melanocytes of white human skin or of the so-called 'albino' guinea-pig rather than with the non-pigmentary melanocytes of, for example, the white areas of recessively spotted guinea-pigs (Billingham, 1948).

The overtly pigmented and Dopa-positive melanocytes are essentially similar to those described by Billingham (1948) in the guinea-pig. Their cell bodies or perikarya lie at the lowest level in the epidermis and frequently abut into the corium. Each gives rise to three to six dichotomizing branches, some of which extend for  $10-30\mu$  along the dermo-epidermal interface before turning outwards to branch among the Malpighian cells (Pl. 1, figs. 6-9). The finest branches of the melanocytes end in tiny button-like caps which are applied to the surfaces of adjacent Malpighian cells. These Malpighian cells are often more heavily loaded with melanin than the melanocytes themselves; but inasmuch as the melanocytes are the only Dopa-positive cells in the epidermis, and epidermal pigmentation is never found in their absence, it is generally agreed that they are the only site of melanin synthesis. There is convincing evidence (Masson, 1948; Billingham, 1948) that pigment granules manufactured within melanocytes pass via the end caps into the surrounding Malpighian cells, so accounting for the polar distribution which the melanin inoculum first assumes—a mode of activity described by Masson as 'cytocrine'. As pigmented Malpighian cells pass towards the skin surface in the course of desquamation, the melanin within them becomes more diffusely distributed.

The unpigmented melanocytes belonging to the same anatomical system as the actively pigment-forming melanocytes are virtually indistinguishable from the latter except in being Dopa-negative and containing no melanin granules, or, at most, very few.

'Ghost' cells. The supra-vital staining of split skin with brilliant cresyl blue shows that dendritic cells are not confined to the basal layer of the epidermis. With certain qualifications, to be mentioned below, all the epidermal epithelia which contain a system of melanocytes in the basal layer also contain a system of branched cells at more superficial epidermal levels (Pl. 2, figs. 12, 13). These 'high-level' branched cells are most specifically revealed by the impregnation of thin shavings by Gairns's gold chloride method (Pl. 2, figs. 14, 15). High-level branched cells are similar in general shape, number and distribution to the melanocytes of the basal layer, but they are more deeply stained by brilliant cresyl blue, their cell bodies are more angular and wiry in appearance, and their nuclei are less easy to distinguish. The branches that arise from them are better revealed by gold impregnation than by supra-vital staining. A full investigation by Billingham & Medawar (1953) has confirmed Masson's (1948) hypothesis that these high-level branched cells are expended, effete melanocytes passing outwards in the course of being desquamated at the skin surface—a hypothesis supported by their similarity of shape, number and distribution, by their position in the epidermis and their failure to give any evidence of mitotic activity. As in the guinea-pig and in man, these high-level melanocyte 'ghosts' do not contain melanin and are never Dopa-positive. It is therefore presumed that they discharge their pigment completely before passing outwards through the epidermis.

#### *Skin of different regions*

*Ear.* The skin of the dorsal surface of the ear can be seen to be deeply pigmented around the margins and to become progressively paler towards the base. On splitting the skin it is immediately apparent that most of the visible pigment is situated

in the corium, which has not been made the subject of a special investigation. Dopa-stained preparations of sheets of epidermis prepared from the dorsum of the ear reveal that active pigmentary melanocytes are very abundant immediately around the ear margin and in an area just behind the medial margin (Pl. 1, figs. 6, 8, 9), but away from the margins they occur only singly or in isolated groups, and they are totally absent from the base of the ear. On the ventral surface of the ear the distribution of active melanocytes differs only in that most of them are situated just within the outer rather than the medial margin.

Supra-vitally stained preparations reveal that melanocytes are in fact evenly distributed throughout the epidermis (Pl. 2, fig. 12). Where active Dopa-positive melanocytes are sparse, non-pigmented melanocytes are seen to be interspersed among them. Some of the inactive melanocytes which occur in close proximity to the pigmented cells can be seen on closer observation to contain a few fine melanin granules. At the base of the ear only non-pigmented dendritic cells occur.

*Tail.* The skin of the tail appears to be made up of a system of slightly raised rectangular blocks which are arranged in annuli around the long axis. Each of these blocks is deeply pigmented, but between them there occurs a very narrow zone of non-pigmented epidermis (Pl. 2, fig. 16). The hair follicles, which always open on to the surface of the epidermis in the non-pigmented zones between adjacent annuli, can be seen to be grouped in threes, each triad corresponding to one pigmented block (Pl. 1, figs. 4, 5). Dopa-stained preparations of tail skin epidermis show that, as the pattern of pigmentation suggests, the active melanocytes are concentrated into more or less isolated blocks measuring approximately 425 by 450  $\mu$ . Each block contains about sixty Dopa-positive dendritic cells, and the surrounding Malpighian cells are densely pigmented (Pl. 2, fig. 11). Only rarely can active melanocytes be distinguished in the narrow zones between the blocks.

Brilliant cresyl-blue preparations show that the non-pigmented epidermis between the blocks of pigmented epidermis contain abundant 'ghost cells' (see above). Such preparations failed to reveal 'ghost cells' in the more superficial epidermal strata of pigmented regions, but this was evidently because the density of pigmentation in the basal layer made the preparations too opaque, for gold-impregnated material showed that 'ghost cells' were evenly distributed through the epidermis, irrespective of its degree of pigmentation. A situation in which 'ghost cells' occur in regions of the epidermis from which basal layer melanocytes are absent is at first sight inconsistent with the idea that the latter are their precursors. In an analysis of an exactly comparable case in the guinea-pig (where 'ghost cells' but not melanocytes are found in the thin, mitotically inactive 'valleys' of trunk skin epidermis between the thick and boldly pigmented 'hills'), Billingham & Medawar (1953) gave strong evidence for believing that there was a constant displacement of epidermal cells from the thicker into the thinner regions of the epidermis. In effect, the thin regions of the epidermis contain cells corresponding anatomically and chronologically to those which, in thicker regions, occur in relatively superficial strata. It is very likely that this is also true of the epidermis of the mouse's tail, for the epidermis is much thinner in the interstices between the pigmented blocks than in the blocks themselves; and it is relevant that Kiil (1949) has demonstrated a constant migration of epidermis over the underlying tissues towards the tip of the rat's tail.



*Sole-of-foot.* This is normally hairless and usually appears greyish in colour, but, as in ear skin, most of the visible pigment is contained in the corium. The distribution of active pigmented melanocytes in the epidermis varies widely from animal to animal. There are usually a few scattered round the lateral margins of the foot and often one or two aggregates elsewhere, but they are never abundant. Inactive melanocytes can, however, be revealed by brilliant cresyl blue wherever they are not replaced by black ones, and 'ghost cells' are evenly distributed throughout the epidermis (Pl. 2, figs. 14, 15).

*Trunk.* Brilliant cresyl-blue treatment shows that dendritic cells form a complete network throughout the surface epidermis of body skin. Owing to the extreme thinness of the epidermis (Pl. 1, fig. 3), dendritic cells and 'ghost cells' can be seen in almost the same focal plane (Pl. 2, fig. 13). Dopa-positive cells have not been demonstrated in normal body skin.

To determine whether the dendritic cells of body skin are of the non-pigmentary or potentially pigmentary type, areas on the side of the thorax of agouti mice were shaved and then painted every 3 days with a mixture of equal parts of turpentine and acetone. This mixture induces epidermal hyperplasia (Friedewald, 1942; Rous, 1946), and since the general cellular activity was greatly increased it was considered likely that, if the dendritic cells were of the potentially pigmentary type, they might be induced by this treatment to elaborate melanin. After about 3 months the mice were destroyed and the epidermis from treated areas examined. In unstained sheets of pure epidermis a few very feebly pigmented dendritic cells were seen scattered throughout the basal layer. With Dopa treatment, however, these cells gave a strongly positive reaction and their processes could be followed between the pigment-capped Malpighian cells. This result was considered to be a sufficient indication that the dendritic cells present in the epidermis of body skin of agouti mice are capable of melanin production. That they are normally Dopa-negative is possibly due to the fact that they are normally under the influence of an inhibitor of melanogenesis (see Discussion).

*Dendritic cells in the hair bulbs.* The stage of growth of a hair can be determined approximately by the position of its bulb in the corium. In skin in which the hairs are regenerating, the bulbs lie deeply embedded in the comparatively thick layer of adipose tissue which lies between the corium and the panniculus carnosus. As the hairs become fully grown the adipose tissue gradually disappears and the bulbs come to lie within the corium (see Durward & Rudall, 1949, who report similar findings for the rat). Dopa-positive melanocytes can be distinguished only in the bulbs of growing hairs, but they leave a record of their activity in the pigmented part of the shaft of the agouti hair. In fully formed hairs the dendritic cells contain little or no melanin and the Dopa reaction is negative.

When skin which bore fully grown hairs was split by the tryptic digestion method, a number of complete hair follicles came away with the epidermis. Supra-vitally stained preparations of this material revealed inactive melanocytes in the matrices of these follicles, and occasionally inactive melanocytes and ghost cells could be seen among the epidermal cells of the outer root sheath. These results suggest that the dendritic cells of the hair matrix produce melanin and distribute it to the germinal cells until the hair is full grown. Afterwards they remain quiescent, and

their melanogenic enzyme complex becomes attenuated or inhibited in its action until a new hair is about to be produced (see Taylor, 1949).

In order to determine the way in which dendritic cells reach the matrix of hair bulbs, a brief study was made of the skin of newly born mice. In lightly carmalum-stained sheets of epidermis prepared from the skin from the backs of day-old mice, small mounds of cells which represent the rudiments of hair follicles can be seen (Pl. 2, fig. 10). These are arranged in roughly parallel rows of about nine, each row normally including one fully formed guard hair (primary hair) follicle. Among the cells of the hair rudiments and in the superficial epidermis immediately surrounding each, a few very feebly pigmented melanocytes could be distinguished. In supra-vitally stained preparations, these pigmented cells could be seen to lie within a network of mostly pigment-free melanocytes which spreads throughout the surface epidermis and that of the hair follicles. With Dopa treatment the pigmented melanocytes gave a strongly positive reaction, but in addition, many of the apparently pigment-free melanocytes, both in hair rudiments and in the surface epidermis around them, were found to be weakly Dopa-positive.

Similar examination, on successive days, of mice from the same litter showed that strongly Dopa-positive cells gradually assumed an active melanogenic state. By the fourth day most of the hairs had begun to penetrate the surface of the epidermis. At this stage there is a dense network of active melanocytes in each hair bulb, though they are still abundant in the necks of the follicles and in the surface epidermis surrounding the follicle openings. Henceforth, however, Dopa-positive melanocytes of the surface epidermis decreased in number, and, by about the 12th day, pigmented dendritic cells were confined to the matrices of the hair bulbs.

*Vaginal epithelium.* Sections of the vaginal mucosa show that the epithelium forms characteristic inwardly directed 'pegs' which alternate with papillary elevations of the corium (Pl. 1, fig. 1). The mucosa was difficult to split by the trypsin method, and separation was successfully achieved only with immature mice. Whole-mount preparations of such sheets of epithelium show a distinct 'hill and valley' pattern. Although brilliant cresyl-blue selectively stains dendritic cells over the whole of the basal layer, they are most abundant over the crests of the ridges. This is the general rule with thick epithelia (Billingham & Medawar, 1953). Ghost cells were clearly visible in superficial strata of the epithelium. No trace of pigmentation in vaginal epithelium was found in any of the animals examined.

*Tongue epithelium.* Thin shavings from the dorsal surface of the tongue (Pl. 1, fig. 2) were split by the trypsin method and the sheets of epithelium derived from them were supra-vitally stained with brilliant cresyl blue. The stain selectively revealed occasional aggregates of bipolar cells having a single fine process at each end, but there was no evidence of the presence of dendritic cells or 'ghost cells.' It was assumed that the bipolar cells were sensory elements associated with the sense of taste and which are known to stain supra-vitally with methylene blue. These results conform with those of Billingham & Medawar (1948), who failed to demonstrate dendritic cells in the tongue epithelium of guinea-pigs.

(2) *Black-and-white recessively spotted mice*

A survey of the distribution of dendritic cells was carried out exactly as described for agouti mice. It is convenient here to deal separately with the findings for the black and the white areas on the mouse.

*Black areas.* In any area included in a black spot, the distribution of active melanocytes was found to correspond exactly with the situation in agouti mice, i.e. they are only to be found in hair follicles and the surface epidermis of the tail, the margins of the ears and the sole of the foot. In body skin, and over the greater part of the ears and sole, inactive melanocytes only are present. These could be specifically stained with brilliant cresyl blue.

When black hair bearing regions of the body were painted with turpentine and acetone the results were similar to those obtained with agouti mice, and the dendritic cells of these areas are therefore concluded to be of the pigmentary type—they are facultatively melanogenic.

*White areas.* Pigmented melanocytes were found to be totally absent from the epidermis of white areas. In supra-vital stained preparations it was often impossible to distinguish dendritic cells in the basal layers of the epidermis. They were very feebly stained in ear and body skin epidermis, but were not revealed at all in the tail and sole of the foot. However, it was inferred that dendritic cells were present in these areas since 'ghost cells', presumed to be their desquamation products, were clearly revealed in the superficial layers of the epidermis by supra-vital staining. Further evidence in support of their presence derives from the fact that pigment spread occurs when white skin is adjacent to black in the tail (see below).

When white-skin areas on the body were treated with turpentine and acetone, there was no sign of pigmentation, and even after prolonged treatment with this and other hyperplasia-inducing agents the results were consistently negative. These results conform with the findings of Lewin & Peck (1941) and Ginsburg (1944) for the white-skin areas of black-and-white spotted guinea-pigs. It is concluded that the dendritic cells in white spots are of the non-pigmentary type, lacking the necessary enzyme system for melanin formation.

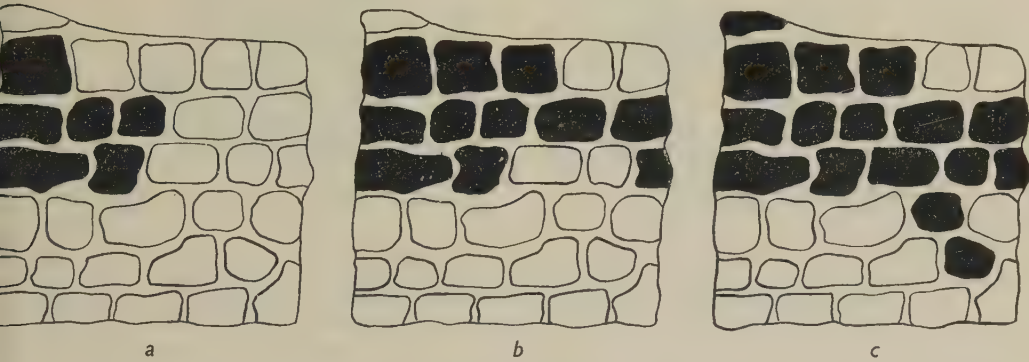
'*Pigment spread.*' In the skin of spotted black-and-white guinea-pigs pigmentation encroaches progressively upon the adjacent non-pigmented epidermis. Billingham & Medawar (1948, 1950) have proposed that pigment spread is the consequence of a propagated cellular transformation. Briefly, their hypothesis is as follows: where coloured and white-skin areas meet, the finest branches of the melanocytes in the coloured skin may make cytoplasmic contact with non-pigmentary dendritic cells present in white skin. Through these anastomoses some cellular ingredient essential for the production of melanin is passed from the cytoplasm of the pigmented melanocytes into their unpigmented neighbours, so endowing them with the faculty of melanogenesis. These 'infectively' transformed cells are then in their turn able to transform further dendritic cells in the white skin with which they are in contact.

Since there are pigmentary dendritic cells in the surface epidermis of the black areas on the tails of spotted mice, it seemed likely that pigment spread might occur where white skin is adjacent to black. To investigate this possibility, the technique of skin transplantation was employed, since this is known to expedite pigment



spread where it normally occurs (see Billingham & Medawar, 1948). Using the standard operative procedure described by Billingham & Medawar (1951), grafts of tail skin, each including both black and white areas, were transplanted to white recipient areas on the side of the animals' chests. Each graft was very thin and rectangular in shape (approximately 4 by 3 mm.), and was cut so as to include mainly white skin with a small area of black.

Six weeks after grafting macroscopic and microscopic examinations revealed that the grafts had retained the distinctive characteristics of tail skin. The pigmented areas of the grafts were almost doubled at the expense of the non-pigmented, though this encroachment of pigment did not involve the surrounding white trunk



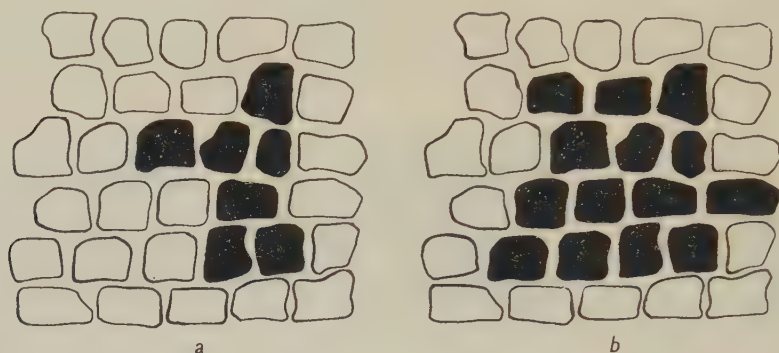
Text-fig. 1. Successive stages in the process of 'pigment spread' in a black-and-white tail skin graft transplanted to a white area on the chest of a spotted mouse, (a) 2 weeks after transplantation; (b) after 6 weeks; (c) after 32 weeks.

skin of the recipient area. During the following 2 months the rate of spread decreased to the average rate of one pigmented block in about 6 weeks (see Text-fig. 1). Pigment spread was most rapid in the lateral direction (around the ridges or annuli of the tail rather than down its long axis). This coincided with an anatomical observation made on sheets of split tail skin—connexions were sometimes seen between the melanocytes of one pigmented block and another in the same annulus, but only very seldom between melanocytes in blocks of different annuli. It was observed that, as in guinea-pigs, hairs on secondarily pigmented zones remained white.

The rate of pigment spread under natural conditions was observed by studying the black spots which sometimes occurred near the tips of the tails. At the time of the first inspection the animals were not quite fully grown. Pigment spread was found to take place very slowly for about 2 months while the animals were still growing, but after that it ceased completely (Text-fig. 2). The application of turpentine and acetone considerably enhanced the rate of spread. In several mice pigment had spread after 3 months' treatment from a single isolated block to the five or six adjacent blocks. Although painting was extended over the white skin well beyond the pigmented areas, no pigmented spots arose spontaneously.

The failure of supra-vital dyes, such as methylene blue or brilliant cresyl blue, to

reveal the living dendritic cells whose existence has been inferred in the basal layer of the epidermis of white body skin from recessively spotted mice, may be attributable to their lack of oxidase enzyme systems. Living pigmentary dendritic cells, whether actively or potentially pigmentary, which can be so revealed, presumably because the dyes are kept in an oxidized state, are known to possess oxidase enzyme systems believed to be responsible for their melanogenic properties.



Text-fig. 2. Natural pigment spread arising from an isolated spot of pigmentation near the tail tip of a black-and-white mouse. (a) on first observation; (b) 2 months later. Observations were continued for a further 6 months, but no further spread occurred.

#### DISCUSSION

It has been demonstrated in this investigation that the epidermis of mouse skin contains a network of dendritic cells which closely resembles that of the guinea-pig and man (Billingham, 1948, 1949; Billingham & Medawar, 1953). The dendritic cell system of the mouse has been found to extend throughout the epidermis of the entire body. It is also present in the vaginal epithelium, but absent from the epithelium of the tongue. The density of distribution of dendritic cells (irrespective of their pigmentary activity) appeared to be the same whether the coat of the mouse was agouti or spotted black and white. The existence of a comparable density of dendritic cells in the epidermis of the white spots of spotted black-and-white mice is inferred from the incidence of their effete end products in the high levels of the epidermis.

In agouti mice it has been shown that all of the melanocytes are of the pigmentary type, i.e. they are either actively or potentially melanogenic. The melanocytes throughout the surface epidermis of trunk skin are normally unpigmented, but if this epidermis is rendered hyperplastic by treatment with turpentine and acetone they become actively melanogenic. In the ears and sole of the foot, in which a minority of the melanocytes is normally Dopa-positive or overtly pigmented, there can sometimes be seen a gradation in melanin content from the white to the pigmented dendritic cells.

For the skin of albino guinea-pigs (Ginsburg, 1944), and of the 'dominant' white spots on English rabbits (Onslow, 1915), it has been suggested that although the

enzyme system necessary for melanin production is present, an inhibitor is also present in the skin. Rothman, Krysa & Smiljanic (1946) have reported that sulphhydryl compounds, which are known to inhibit melanin formation *in vitro*, are present in white human skin. It is conceivable that a similar inhibitory system is at work in the non-pigmented epidermis of agouti mice, and that it is counteracted by a treatment which causes hyperplasia in a manner analogous to the action of ultra-violet irradiation on white human skin. An alternative possibility is that such melanocytes normally contain insufficient of the enzyme complex necessary for active melanogenesis and that treatment with hyperplasia-inducing agents causes more to be formed.

Rawles (1947) has shown that the 'melanophores' (here 'melanocytes') of the hair follicles of mice derive from the neural crest of the embryo, and that they are present in all presumptive hair matrices by the twelfth day of gestation. In the present investigation the results of observations made on the skin of newly born mice indicate that melanocytes of the presumptive hair follicles form part of the same system as those which belong to and remain within the rest of the epidermis. As the follicles begin to penetrate into the corium, melanocytes which retain melanogenic properties are carried down into the hair bulbs. It thus appears that although the melanocytes of the hair follicles eventually form a distinct anatomical subsystem, the epidermal system as a whole originates from the same source. Even in adults, Billingham & Medawar (1953) have shown that the melanocytes of the hair bulbs and of the surface epidermis are experimentally interchangeable.

Rawles (1947) believes that the melanocytes of the hair matrices degenerate as the hairs begin to keratinize, and that new ones are continually being supplied at the growing bases to provide pigment for additional parts of the hairs as they differentiate. She suggests that each new generation of hairs receives a fresh complement of melanocytes via the dermal papillae. This, however, seems unlikely, for by means of the Dopa technique, pigmentary melanocytes can be distinguished in the matrices of all developing coloured hairs, and although the melanocytes lose their pigment by the time the hair is fully grown, they are still visible after supravital staining with brilliant cresyl blue. Perhaps the most convincing explanation for the reappearance of pigmented melanocytes in regenerating hair follicles has been given by Taylor (1949). He suggests that the melanocytes remain quiescent in the matrices of the fully grown hairs until new hairs are about to be formed. Some then migrate with the other cells which form the new hair primordia, multiply and become melanogenic.

Apart from the streaming movements of the melanocytes together with other cells into the developing hair follicles, I have found no evidence for the migration of dendritic cells within the epidermis. Reed (1938) and Reed & Henderson (1940) believe that melanocytes invade skin grafts transplanted from one new-born mouse to another, the pigment cells of the donor combining with the hair germs of the host. These findings, however, appear to apply only to the line of suture between the grafts and the surrounding tissue, where special factors are clearly at work.

The view that melanocytes are the malignant elements in melanotic tumours of the skin is steadily gaining ground. There are at least two known transplantable mouse melanomas, and anatomical and biochemical investigations have produced



strong evidence that they are in fact tumours of melanocytes (Grand, Chambers & Cameron, 1935; Grand, 1938; Hogeboom & Adams, 1942; Woods, Du Buy, Dean Burk & Hesselbach, 1949). The present paper is relevant to this problem, in so far as it has been shown that melanocytes in mice are present throughout the epidermis and are not confined to the hair bulbs, as was previously thought.

#### SUMMARY

1. Melanocytes are a constant ingredient of the epidermis of mice irrespective of whether it is pigmented or not. They are also present in the epithelium of the vagina, but not in that of the tongue.

2. All the melanocytes of agouti mice are of the pigmentary type. Only the melanocytes of the relatively hairless regions and the hair bulbs normally manufacture and contain melanin, but those of the rest of the body can be induced to do so if the skin is rendered hyperplastic.

3. In black and white 'recessively-spotted' mice pigmentary melanocytes are confined to the black regions, but, as in agouti mice, only a few are normally melanogenic. Dendritic cells in recessive white spots whose existence is inferred from the presence of their desquamation products, are neither actively nor potentially pigmentary, and no known stimulus will cause them to give a positive Dopa reaction or to elaborate melanin.

4. It is suggested that melanocytes are constantly present in the bulbs of pigmented hairs, but they enter a quiescent, inactive phase after the hairs are fully grown.

5. Observations on the development of hair follicles in newly born mice suggest that as the follicle rudiments penetrate into the corium, they carry down with them a complement of melanocytes from the system already present throughout the surface epidermis. The remainder persist as the melanocyte system of the surface epidermis of the adult.

6. Pigment spread, i.e. the encroachment of pigmentation from black into neighbouring white areas, has been observed to take place on the tails of spotted mice.

7. Evidence is presented to show that effete melanocytes pass outwards, like Malpighian cells, through the epidermis to be desquamated at the skin surface.

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## EXPLANATION OF PLATES

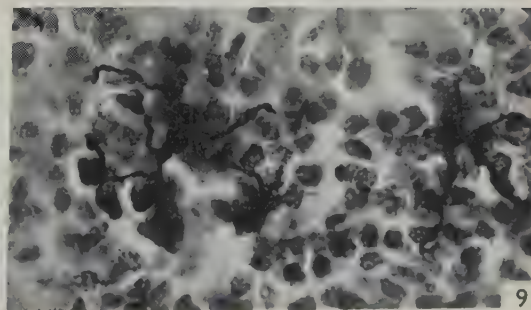
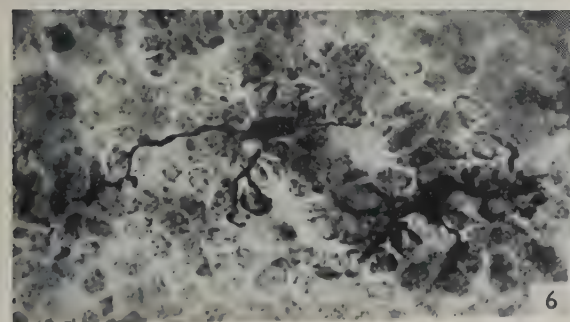
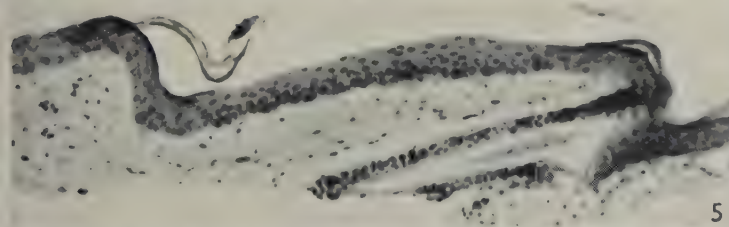
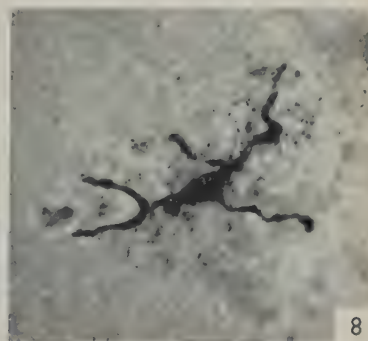
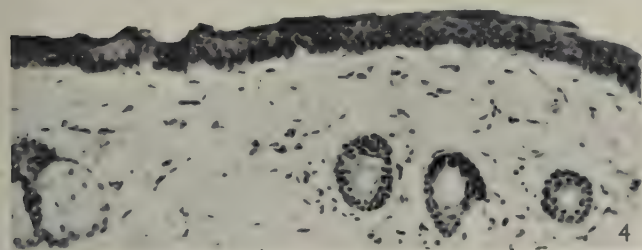
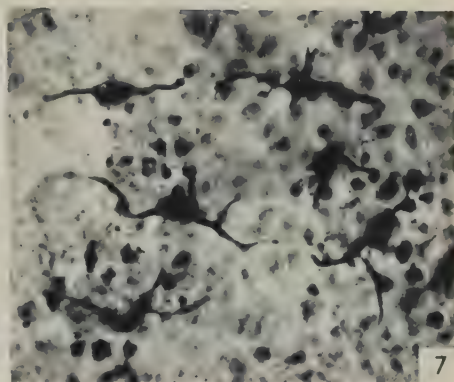
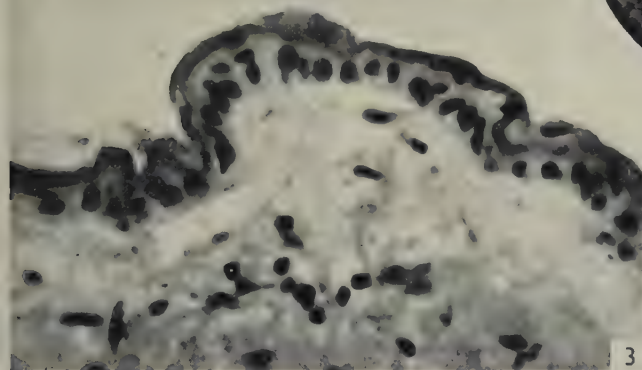
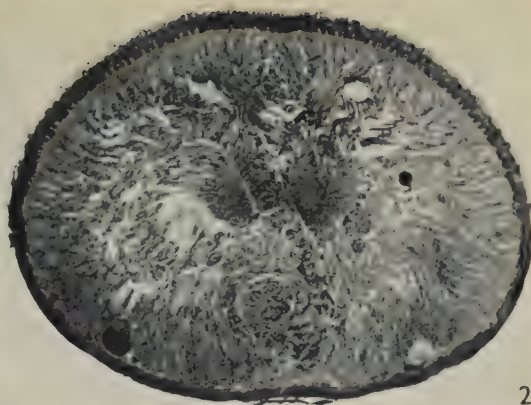
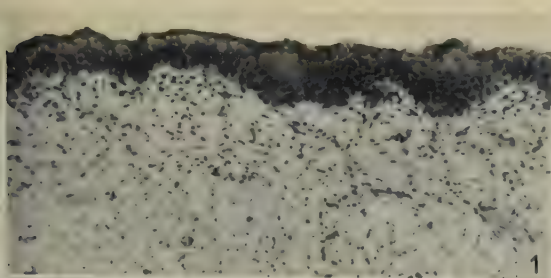
## PLATE 1

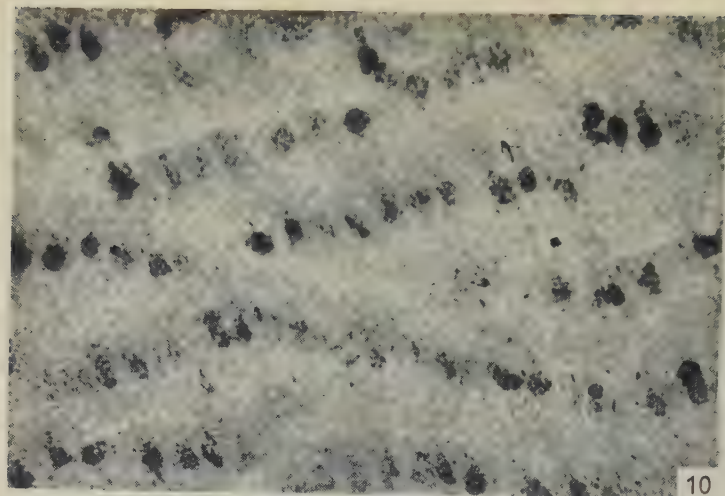
- Fig. 1. Vertical section through the vaginal epithelium of an agouti mouse, showing the irregularity of the inner surface of the epithelium. Ehrlich's haematoxylin and eosin.  $\times 126$ .
- Fig. 2. Vertical section through the tip of a mouse's tongue. The epithelium of the dorsal surface penetrates the underlying connective tissue in regular 'pegs'; that of the ventral surface is comparatively flat.  $\times 20$ .
- Fig. 3. Vertical section through the trunk skin of an agouti mouse: note the delicacy of the epithelial layer, which consists of a single layer of generative cells invested by a thin cuticle. Ehrlich's haematoxylin and eosin.  $\times 520$ .
- Figs. 4, 5. Transverse (fig. 4) and sagittal (fig. 5) sections through the skin of a mouse's tail, showing the disposition of the triad of hairs in relation to the epidermal blocks (see Pl. 2, fig. 16), which give the sagittal section its imbricated outline. The epidermis is much thicker than that of body skin (Pl. 1, fig. 3). Ehrlich's haematoxylin and eosin.  $\times 70$ .
- Figs. 6-9. Actively melanogenic melanocytes in sheets of pure epidermis ('split skin') which have been treated with the Dopa reagent (agouti mice); figs. 6, 8, 9 are of ear skin, fig. 7 is tail skin. The preparations are photographed from their inner (dermal) surfaces. The cell bodies and branches of the melanocytes have been deeply blackened by the reagent; each melanocyte is surrounded by a cluster of Malpighian cells heavily inoculated with melanin granules.  $\times 520$ .

## PLATE 2

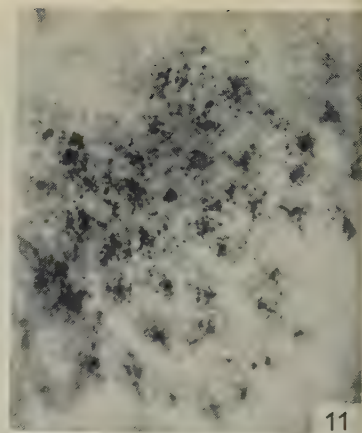
- Fig. 10. A whole mount of the epidermis from the trunk-skin of a day-old agouti mouse, treated with the Dopa reagent and lightly stained with carmalum. Note the presence of both strongly and weakly Dopa-positive melanocytes within and around each follicle rudiment.  $\times 126$ .
- Fig. 11. A whole mount representing a single pigmented block of tail skin epidermis (cf. Pl. 2, fig. 16) after treatment with the Dopa reagent. Individual Dopa-positive melanocytes are clearly discernible; the Malpighian cells around them are heavily inoculated with pigment granules.  $\times 126$ .
- Figs. 12, 13. 'Split' epidermis of ear skin (fig. 12) and trunk skin (fig. 13) (agouti mice) stained supra-vitally with brilliant cresyl blue and photographed from the inner (dermal) side. The dendritic cells seen in fig. 12 are mostly inactive, pigment-free pigmentary melanocytes, anatomically equivalent to those illustrated by Pl. 1, figs. 6-9, but 'ghost cells' can be indistinctly seen in a deeper focal plane. In trunk skin (fig. 13) the two types are hardly distinguishable in a photograph.  $\times 520$ .
- Figs. 14, 15. 'Ghost' cells in sole-of-foot epidermis from an agouti mouse, revealed by a variant of Gairns' gold-impregnation method. Compare with fig. 13: gold-impregnation reveals cell processes much more distinctly than supra-vital staining with brilliant cresyl blue.  $\times 520$ .
- Fig. 16. A low-power photograph, taken from the inner (dermal) side, of pure epidermis from the tail skin of an agouti mouse: the antero-posterior axis of the tail runs from left to right. Note the pigmented blocks of epidermis arranged in coaxial rings. Cf. Pl. 1, figs. 4, 5.  $\times 20$ .



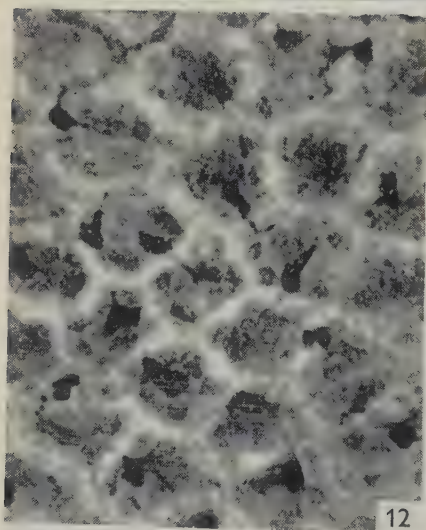




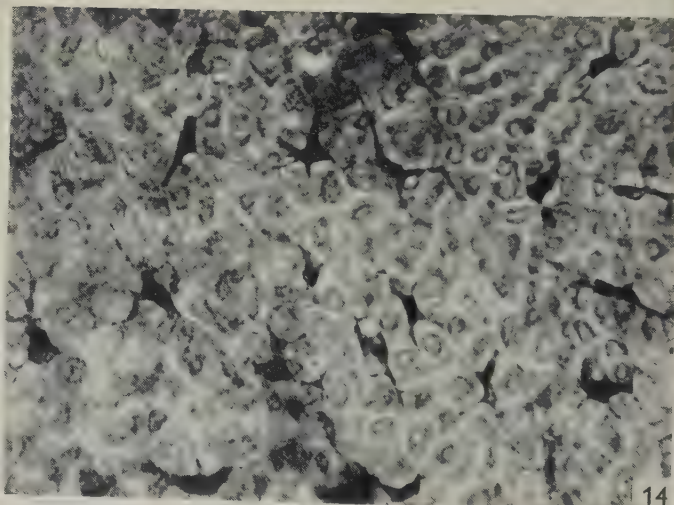
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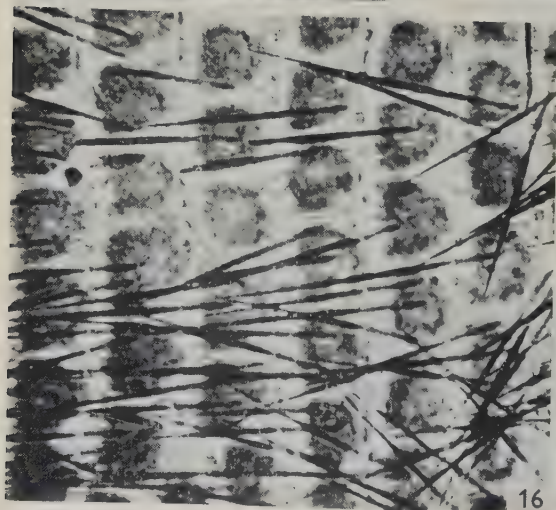
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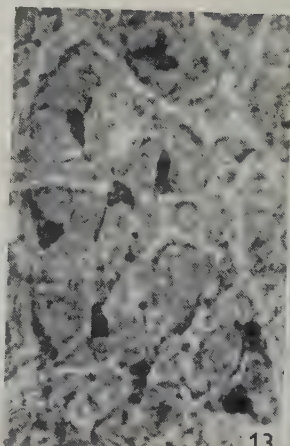
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# A COMPARISON OF THE HUMAN KNEE AND AVIAN ANKLE

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The manner in which the main articulations of the vertebrates have become variously modified in relation to diverse function has been investigated by many workers, notably Owen (1866), Parsons (1900) and Haines (1942). Relatively few have pursued the converse study—the elucidation of the remarkable similarities often exhibited by vertebrate joints that are in no way homologous. Yet such an investigation might well shed light upon the complex problem of the mechanics of joint movement, for resemblances in joints that are analogous but not homologous must necessarily have arisen independently in association with similar functional requirements.

In the present investigation, a detailed comparison is made between two analogous joints, the human knee and the so-called ankle of the turkey, with the object of determining similarities of structure and thus of function. The latter joint is chosen as an example of a bicondylar ginglymus joint possessing intra-articular fibrocartilages, capable of a wide range of flexion and extension, and exhibiting also some axial rotation. It is situated at the junction of the two main long bones of the hind-limb, the tibia (more correctly, tibiotarsus) and the tarsus (tarso-metatarsus). The general form is very similar to that of the human knee: a pair of rounded condyles at the lower end of the tibia articulate with two articular facets at the proximal end of the tarsus, upon each of which rests a semilunar fibrocartilage.

At both joints special attention has been paid to the form and attachments of the intra-articular menisci, which have been credited with the function of ensuring efficient lubrication throughout joint movement (MacConaill, 1932), and to the ligaments, the function of which is disputed (Partridge, 1924). The surrounding muscles and tendons, which undoubtedly play an important part in stabilizing these joints, are not described in detail.

## MATERIAL AND METHODS

Twelve freshly-amputated human knees have been dissected; in addition the contours of a number of cartilage-covered bones obtained from cadavers have been measured. The knee-joints of several other mammals (e.g. macaque, dog, fruit-bat, rat and rabbit) have also been studied, confirming that in most respects the human knee is typical of the eutherian mammals. Eight ankle-joints of turkeys have been examined; previous study of the ankles of the pigeon and fowl had indicated that these were too small to allow accurate dissection of the intra-articular structures. In all joints the skin was removed and the range of movements then tested. Subsequently the collateral ligaments were carefully dissected and note was made of their tension at different phases of flexion and extension. These ligaments were



then cut one by one, the increased amount of passive movement that resulted was noted, and finally the intra-articular structures were examined.

The contours of the bones were measured either by sawing them through and then tracing their cut outlines, or by matching their curves with circles of known diameter etched on sheets of 'Perspex'.

Cineradiography of the human knee and slow-motion cinephotography of walking birds (fowl and pigeon) have also been used to investigate the movements of the joints.

### OBSERVATIONS

The similarity between the two joints can be appreciated only if it is realized that they are transposed mirror images of each other. Thus the front of the human knee is comparable to the back of the turkey's ankle, and the medial surface of the knee resembles the lateral surface of the ankle. The angle between the tibia and tarsus of the turkey is open anteriorly, so that the flexors lie in front of the ankle and the extensors behind, in contrast to the human knee.

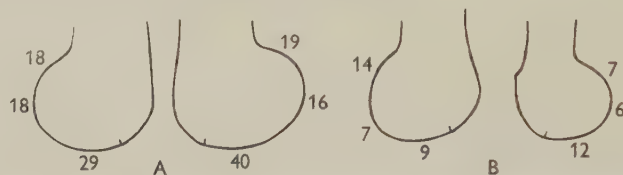


Fig. 1. A, the profiles of the medial and lateral femoral condyles (human, left knee); B, the profiles of the lateral and medial tibial condyles (turkey, left ankle). The radius of curvature, in mm., is indicated in three regions on each condylar profile.

### Articular surfaces

In the human knee, the profiles of the femoral condyles are usually said to be spiral in form, their curvatures gradually increasing in diameter from back to front. The lateral condylar profile may be described more accurately as a combination of two spirals: it is highly curved in the intermediate portion and flattens towards either extreme (Fig. 1A). The form of the medial condyle is similar, but the change in curvature towards the back of the condylar profile is usually very slight, and this part of the curve commonly forms an arc of a circle.

In the ankle joint of the turkey, the profiles of the tibial condyles resemble those of the human femoral condyles, but the changes in curvature are usually more obvious (Fig. 1B).

Measurements of the curvature of the articular surfaces in the *coronal* plane have been carried out at various points on each femoral condyle, by laying a straight-edge across the undersurface of the two condyles, marking the points of contact, and then determining the curvature in the region of these points by means of matching curves of known radius. In cartilage-covered specimens, the curvature measured in the coronal plane is found to be inversely proportional to that in the sagittal plane, except in the most anterior part of the femoral condyles. A typical set of measurements is shown in Table 1. The significance of these findings will be mentioned in the discussion. A similar relationship appears to exist at the turkey's ankle, but the tibial condyles are too small to allow accurate measurements of the curvature in the coronal plane.

The long axes of the femoral condyles in man are not parallel to one another except in their middle thirds (Fig. 2A). Anteriorly, the medial condyle curves towards the lateral (Meyer, 1853); posteriorly, the lateral condyle curves somewhat medially (Goodsir, 1868). In the turkey's ankle, on the other hand, the tibial condyles are set close together in their middle thirds, diverging markedly from each other anteriorly and to a lesser extent posteriorly also (Fig. 2B).

Table 1. *The radii of curvature of the human femoral condyles*

Condyle	Where curvature measured	Radius of curvature in sagittal plane (cm.) $r_1$	Radius of curvature in coronal plane (cm.) $r_2$	Anatomical curvature $\left(\frac{1}{r_1} \times \frac{1}{r_2}\right)$
Lateral	Near front	4.5	2.8	0.079
	Near back	2.2	5	0.091
Medial	Near front	5	2.3	0.087
	Near back	2.5	5	0.080

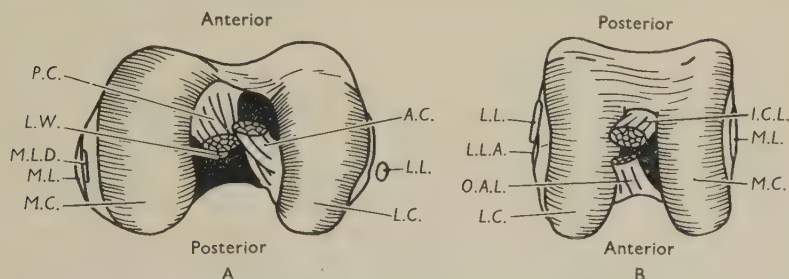


Fig. 2. A, the articular surfaces of the femoral condyles (human, left knee); B, the articular surfaces of the tibial condyles (turkey, left ankle).

The following abbreviations are used in Figs. 2-4:

A.C.	anterior cruciate ligament	M.C.	medial condyle
B.	bursa	M.L.	medial ligament, principal band
E.T.	extensor tendon(s)	M.L.D.	deep portion of medial ligament
F.P.	synovial fat pad	M.L.O.	oblique portion of medial ligament
I.C.L.	intracapsular ligament	M.M.	medial meniscus
L.C.	lateral condyle	O.A.L.	oblique anterior ligament
L.L.	lateral ligament, principal band	P.C.	posterior cruciate ligament
L.L.A.	lateral ligament, accessory band	P.S.	projection from sesamoid
L.M.	lateral meniscus	S.	ankle sesamoid
L.W.	ligament of Wrisberg		

In the human knee the patella, lying within the main extensor tendon, articulates with the femoral condyles. Below the patella, a large synovial fat pad projects backwards into the femoral intercondylar region, especially in flexion. In the ankle of the turkey there is a triangular sesamoid bone which articulates with the back of the medial tibial condyle. It lies in the lower medial part of a strong retinaculum, within which the extensor tendons occupy separate tunnels. The anterior surface of this retinaculum above and to the lateral side of the sesamoid is covered by a layer of articular cartilage. Below and to the lateral side of the sesamoid is a large synovial fat pad which glides forward between the tibial condyles, overlapping the lateral meniscus, when the joint is flexed.

In the human knee, the underlying articular surfaces are the two tibial articular facets together with the semilunar menisci (Fig. 3A). The medial tibial facet is plane or slightly concave. The lateral is cambered both anteriorly and posteriorly. The posterior horn of the lateral meniscus gains attachment to the intercondylar area of the femur by means of the ligaments of Wrisberg or Humphry. It is attached also to the tibia in man, though not in most mammals (Parsons, 1900). The lateral meniscus is mobile, gliding freely on the tibia; the medial meniscus is relatively immobile.

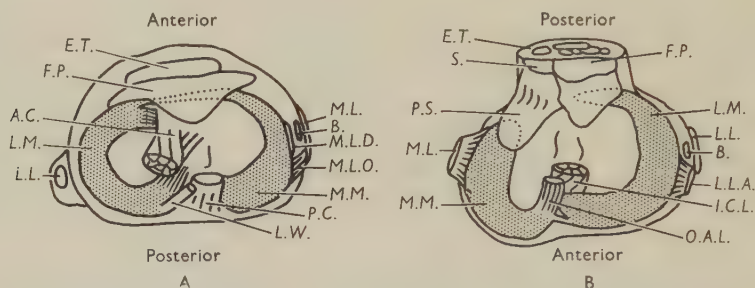


Fig. 3. A, the receiving surfaces for the femoral condyles (human, left knee); B, the receiving surfaces for the tibial condyles (turkey, left ankle).

In the turkey's ankle, the underlying articular surfaces (i.e. the top of the tarsus together with the menisci) are remarkably similar to the tibial plateau in man (Fig. 3B). The upper end of the tarsus bears two articular facets with an intermediate non-articular area. Neither facet is entirely flat—both are cambered anteriorly and the lateral facet is cambered posteriorly also. The lateral meniscus is a large, semilunar fibrocartilage the horns of which are attached to the front and back of the non-articular area of the tarsus. The medial meniscus is smaller. Its posterior horn is attached to the upper surface of the medial tarsal condyle, where it is overlapped by a wedge-shaped projection from the sesamoid bone in the extensor tendon retinaculum. The anterior horn of this fibrocartilage is not attached to the tarsus, but is continuous with a ligament which passes upwards and laterally to gain attachment to the tibial intercondylar region and the medial aspect of the lateral tibial condyle. This stout band, which may be termed the *oblique anterior ligament*, becomes taut when the ankle is extended. A slender ligament runs from the lateral meniscus to the side of the oblique ligament.

#### *Capsule and retinacula*

In both joints the capsule allows a 'suprasedamoid' pouch of synovial membrane to protrude upwards as the joint is straightened. Laterally and medially stout bands of fibrous tissue, the retinacula, extend from the side of the patella (or its equivalent in the turkey's ankle) to gain attachment to both articulating bones, blending with the capsule and usually with the collateral ligaments.

#### *External ligaments*

The *medial ligament* of the human knee (Fig. 4A) has been re-described by several authors (notably Palmer, 1938; Horwitz, 1938; Brantigan & Voshell, 1941; and



Last, 1948). The present findings accord substantially with those of Brantigan & Voshell. Three parts may be distinguished: a principal band, long and flattened, which is separated from the meniscus by a bursa; a thin fan-shaped portion extending backwards from the principal band to gain attachment to the tibia immediately below the articular surface and to the side of the meniscus; finally, a separate, deeper ligament which is essentially a thickening of the true capsule extending between the sides of the femoral condyle and the medial meniscus (Last, 1948). During flexion, the femoral attachment of the principal band moves posteriorly and its posterior fibres slacken. In full extension most of its fibres are taut. The remaining portions of the medial ligament help to maintain the relationship of the meniscus to the femur during axial rotation. They also help to stabilize the fully extended joint.

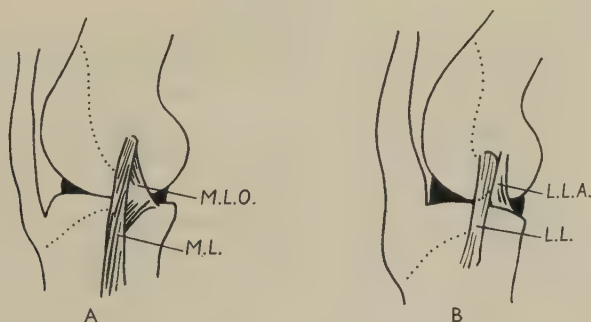


Fig. 4. A, the medial ligaments of the human knee (deep portion omitted); B, the lateral ligaments of the turkey's ankle. Dotted line indicates the attachments of the capsule.

In the turkey's ankle the *lateral* ligament is subdivided into two main portions (Fig. 4B). The principal band is very long and flattened, and is separated from the lateral meniscus by a bursa. Its upper attachment moves anteriorly during flexion; its lower attachment, to the lateral side of the tarsus, is more than  $1\frac{1}{2}$  cm. below the joint line. Few fibres of this band are taut during flexion and even in full extension some are slack. The second portion of the lateral ligament lies anterior to the main band, to which it is loosely connected by fibrous tissue. It is a thickening of the true joint capsule extending between the sides of the lateral tibial condyle and the lateral meniscus, and serves to maintain the relationship of the meniscus to the condyle during axial rotation.

The cord-like *lateral* ligament of the human knee has no connexion with the lateral meniscus. Except in full extension, it is completely lax. During flexion it acts as a check to excessive axial rotation. By contrast, the *medial* ligament of the turkey's ankle is an ill-defined thickening of the capsule, triangular in form, which is fused below to the periphery of the medial meniscus. In full extension all its fibres are taut. It is completely lax during semi-flexion, but in full flexion some of the posterior fibres again tighten.

#### *Intracapsular ligaments*

In the human knee, the twisting of the fibres of the anterior and posterior cruciate ligaments requires mention. The femoral attachments of these ligaments are

adjacent to one another, and their upper parts are sometimes fused. In the turkey's ankle, there is only one intracapsular ligament. This passes upward and backward from the front of the intermediate non-articular area of the tarsus to the intercondylar notch of the tibia. The fibres are twisted so that the anterior group passes to the lateral tibial condyle and the posterior to the medial condyle. Some portion of the ligament is taut in all phases of movement; the anterior portion during extension and the posterior during flexion.

#### *Angulation of the joint*

In man, lateral inclination of the femur on the tibia is greatest when the knee is straightened. Similarly, at the turkey's ankle the tibia is laterally inclined on the tarsus during the last twenty degrees of extension.

#### *Movements*

The movements of flexion and extension at the human knee consist of a combination of *gliding* (successive regions of the upper articular surface making contact in turn with an unchanging portion of the underlying surface) and *rolling* (each region of the upper surface making contact with a corresponding region on the underlying surface, as in a wheel travelling along a road). In full extension there is a minimum of gliding between the lateral condyles but gliding predominates between the medial condyles. Other mammals often show a different type of movement—in the rabbit, for example, it is only during the later phases of flexion that any rolling can be detected.

Flexion and extension at the turkey's ankle show a similar combination of rolling and gliding, but here rolling is the predominant movement during the phase from 60 to 130° of flexion. At both extremes of joint movement there is very little rolling. The relative proportion of rolling and gliding is determined by the tension in the retinacula and the ligaments. Division of the retinacula greatly increases the amount of rolling during flexion.

In the human knee there is free axial rotation in semiflexion. During the terminal phase of extension the femur rotates medially upon the tibia—the 'locking' or 'screwing-home' movement. Once the limb is fully extended no further rotation is possible. At the turkey's ankle a similar mobility is exhibited. When the joint is partly flexed axial rotation is free; in the last phase of extension the tibia undergoes slight lateral rotation; the fully extended limb allows no rotation.

Distraction of the articular surfaces is possible at the human knee except during the last few degrees of extension. At the turkey's ankle a similar passive movement can occur. In addition, a remarkable degree of passive antero-posterior gliding is permitted in all phases save full extension. This mobility is the most striking difference between the two joints.

#### DISCUSSION

The structural similarities present in the human knee and the turkey's ankle are listed below, followed by a discussion of the functional significance of each:

- (a) A lateral inclination of the upper bone upon the lower in the extended limb.
- (b) Free axial rotation in almost all positions of the joint.

(c) Paired semilunar menisci.

(d) A changing curvature of the upper, condylar surfaces, both in the sagittal and coronal planes.

(e) A motion of the condyles during flexion and extension that consists of a combination of rolling and gliding.

(f) Cambering of the front and back of most of the underlying articular facets.

(g) Ligaments that are under greatest tension in full extension (the close-packed position), but parts of which are taut in all phases of joint movement.

The femoro-tibial inclination of the fully extended human knee is presumably associated with the need to maintain the centre of gravity of the body directly above the supporting tibia.

When crouching on one leg, stability can be achieved by rotating the femur medially upon the tibial plateau. Similarly, when the turkey stands on one leg it can maintain the centre of gravity directly above the supporting foot by lateral rotation of the semi-flexed tibia upon the tarsus, probably associated with medial rotation of the femur at the knee, as in the goose (Young, 1950).

Axial rotation is a noteworthy feature in both the human knee and the turkey's ankle. Free rotation is permitted during flexion and is a necessary accompaniment of the terminal phase of extension. The latter 'conjunct' rotation probably serves to maintain a convergent film of synovial fluid between the upper and lower articular surfaces even when these are nearly congruent (MacConaill, 1946).

Intra-articular fibrocartilages were stated by Parsons (1900) to be present in those joints that allow several kinds of movement, especially axial rotation. In support of this view he evidenced the fact that in the knee of the fruit-bat, where axial rotation is impossible, there are no menisci. Conditions in the turkey's ankle reinforce this contention. MacConaill (1932, 1950) concluded, from consideration of the mechanical conditions necessary for perfect lubrication, that fibrocartilages assist the sliding of one flattened surface upon another. The two theories are perfectly compatible, for during axial rotation at bicondylar joints one condyle slides forward upon the corresponding articular facet as the other slides back.

The changing curvature of the condylar profiles is not easily explained, but the disappearance of the wedge-shaped interval between the articular surfaces when the terminal position of the joint is reached probably helps to limit further movement and to stabilize the joint (Walmsley, 1928; MacConaill, 1932). This close-packing of the surfaces is especially important where joint movement cannot be halted by contact between extra-articular structures as, for example, in the position of full extension.

The variation in the curvature of the undersurface of the joint condyles, as measured in the *coronal* plane, is probably necessary to provide efficient lubrication of the articular surfaces. MacConaill (1946) has deduced, on theoretical grounds, that for perfect lubrication in saddle joints the curved path traced out by each moving element must be one of constant anatomical curvature. (The anatomical curvature in the neighbourhood of any point is defined as the product of two curvatures measured in planes at right angles to one another.) The present findings suggest that a similar rule is applicable to bicondylar joints. The products of the curvatures at two points on each of the human femoral condyles are shown in



Table 1, final column. As the tibia moves upon the femur during extension of the knee, one element on each of its surfaces will pass from the posterior to the anterior of the points on the corresponding femoral condyle, tracing out a path every point on which is of constant anatomical curvature.

In both joints, the movement of one surface upon the other involves a combination of rolling and gliding, the rolling element predominating towards the end of extension in the human knee but during most of the flexion range in the turkey's ankle. This difference can be understood by comparing the stresses that these joints are normally called on to withstand. As Steindler (1935) points out, rolling is less likely than gliding to produce attrition of the underlying cartilage-covered facet, since it enables the load to be borne by a different part of this surface in each phase of joint movement. It is therefore not surprising that rolling predominates in the human knee, especially at the lateral condyle, from full extension to  $20^\circ$  of flexion, for this is the phase during which there is greatest pressure upon the joint surfaces in walking or standing. In the turkey, however, the angle at the ankle joint during walking ranges from  $60$  to  $130^\circ$ ; hence it is during this phase that rolling of the tibial condyles is most prominent, minimizing the risk of attrition of the underlying tarsal facets.

Parsons (1900) has noted that the fixation of the back of the lateral meniscus to the tibial plateau distinguishes the human knee from that of other eutherian mammals. A rolling motion involves the travelling of the upper bone forward or backward upon the lower, requiring any fibrocartilage within the joint to move correspondingly. The relative immobility of the posterior part of the human lateral meniscus evidently depends upon the fact that in man, unlike the other mammals examined, there is very little rolling of the femoral condyles during the flexion phase. This limited mobility of the meniscus is therefore directly related to the evolution of an upright posture, the genicular surfaces bearing the greatest load when the knee is nearly fully extended. A comparable example among birds is the Stanley crane (*Tetrapteryx paradisea*), which stands for long periods with fully extended ankles. In this species rolling of the tibial condyles is apparent only during the last few degrees of extension, thus providing a parallel condition to that found in the human knee.

The presence of cambered articular facets on the turkey's tarsus and on the lateral half of the human tibia is related to the backward and forward sliding of the fibrocartilages during rolling of one articular surface upon the other. If a meniscus were to slide on a perfectly flat bony surface and a condyle of changing curvature were to flex and extend in the concavity so formed, the thickness of the wedge-shaped film of lubricant would vary within wide limits. The articular surfaces could even become parallel (Fig. 5A, B) and the efficiency of lubrication would thereby be greatly reduced (Boswall, 1928). It is evidently desirable for the receiving surfaces to become less concave as the entering surfaces increase in diameter, in both joints there exist means of achieving this effect. In the human knee the mechanism is well seen in the lateral condyles (Barnett, 1953); the anterior horn of the lateral meniscus slides down a cambered surface during extension and thus the concavity of the lateral receiving surface is reduced (Fig. 5C). The posterior part of the lateral tibial condyle slopes in a similar way (Fairbank, 1948) so that in the final

stages of flexion parallel moving surfaces are again avoided. The ligament of Wrisberg probably helps to prevent premature close-packing during flexion, for according to Brantigan & Voshell (1941) 'the ligament of Wrisberg, when present, tends to control the lateral meniscus, pulling it backward in flexion'. The lack of a similar mechanism in the medial half of the joint is perhaps due to the fact that the receiving surface (i.e. tibial facet plus meniscus) is much flatter than the condylar profile (Barnett, 1953) so that there is no tendency for the articular surfaces to become parallel until full extension has almost been attained.

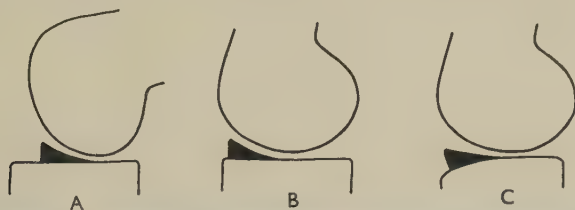


Fig. 5. A, partial flexion: wedge-shaped interval between articular surfaces; B, joint nearly fully extended: surfaces now parallel; C, wedge-shaped interval preserved by cambering of lower surface.

In the turkey's ankle, similar alterations in the concavity of the lateral receiving surfaces are brought about during flexion and extension by sliding of the anterior and posterior horns of the meniscus upon the cambered lateral articular facet of the tarsus. In the medial half of the joint, the oblique anterior ligament relaxes in flexion and allows the anterior horn of the meniscus to glide down the steeply cambered front portion of the tarsal condyle. There is a different mechanism for adjusting the medial receiving surface during extension. The posterior part of this surface is formed by the wedge-shaped projection from the sesamoid bone that lies in the extensor tendon retinaculum. The changing inclination of this retinaculum as the medial tibial condyle is extended causes the sesamoid to be dragged backwards; the projecting wedge of bone is therefore partly withdrawn from the interval between the medial tibial condyle and the underlying articular facet on the talus. As a result the more flattened posterior portion of the tibial condyle can be accommodated.

The ligaments at bicondylar joints certainly help to restrict further movement as the terminal phase is reached, in association with close-packing of the articular surfaces as discussed above. Whether they perform other functions is disputed. Walmsley (1928) maintained that ligaments are taut, and 'therefore functional, in only one position of the joint', and that in all other positions their laxity allows distraction of the articular surfaces. Haines (1944) criticized this generalization, pointing out that many ligaments appear taut in all positions of the joint. The present study indicates that in most ligaments of bicondylar joints a few fibres are taut throughout joint movement. For each position a particular group of fibres will be under tension and the remainder slack. It is likely, therefore, that there is a characteristic pattern of afferent nervous impulses arising in the capsule and ligaments for each position of the joint. These impulses may either reach consciousness as the sensation of proprioception or else serve to initiate reflex contraction of the

surrounding muscles, minimizing strains upon the joint, as suggested by Partridge (1924). Such reflexes would form the physiological basis of Hilton's Law (Hilton, 1892). The question arises: how can distraction of the articular surfaces take place if any parts of the joint ligaments are already under tension? The answer is that those fibres which lie at right angles to the joint line are always slack except in the close-packed position; obliquely disposed fibres may be taut and yet allow distraction, by becoming more nearly perpendicular to the joint line as the bones are drawn apart.

The retinacula, aided in part by the ligaments, are of importance in controlling the type of movement at the joint, especially by determining the relative proportion of rolling and gliding.

*The main differences between the two joints are:*

(a) The distance between the human femoral condyles is greatest at about the junction of middle and posterior thirds, whereas in the turkey's ankle the tibial condyles are furthest apart at their anterior and posterior extremities.

(b) Antero-posterior mobility is a feature of the turkey's ankle but very little is possible in the human knee.

As discussed above, rolling of the condyles converts the weight-bearing region of the underlying surface from a small, circular unchanging area to a narrow strip of the surface disposed antero-posteriorly. The change in distance between the two condyles means that, in gliding of the condylar surfaces, this strip is in effect broadened. In the human knee, for instance, the two regions on the tibial articular facets that bear the pressure of the overlying condyles are set close together in full extension, move apart in mid-flexion and approach one another again in full flexion. The converse changes occur at the turkey's ankle, but the functional effect is similar.

The antero-posterior mobility exhibited in the turkey's ankle is evidently due to the lack of paired cruciate ligaments. It is commonly stated that an important function of these ligaments in the mammalian knee is the prevention of forward and backing gliding of the femur on the tibia, and the present study supports this view. In the living bird, a stable ankle is probably assured by the tone of the flexor and extensor muscles, the multiple tendons of which pass anterior and posterior to the joint, eliminating the possibility of antero-posterior gliding.

#### SUMMARY

A detailed comparison is made between two analogous bicondylar joints, the human knee and the ankle of the turkey, with the object of determining similarities of structure and thus of function. The following conclusions are drawn:

1. Axial rotation can occur at both joints in all phases except full extension (the close-packed position). It is this rotation which calls for the presence of intra-articular fibrocartilages, since it involves the sliding of one flattened surface upon another.

2. The curvature of the under-surface of the condyles, measured in the sagittal plane, varies in such a way that there is least congruity between the articular



surfaces when the joint is partially flexed. The curvature of each condyle in the coronal plane varies in the inverse manner; this relationship between the two curvatures may be necessary to provide efficient lubrication.

3. Since the long axes of the two condyles are not parallel, the distance between the weight-bearing regions on the underlying surfaces changes as the joint is flexed and extended. The likelihood of attrition of these surfaces is thereby reduced.

4. The movement of the condyles is a combination of rolling and gliding, the former predominating during the phase when there is the greatest pressure upon the underlying articular facets.

5. Rolling of the condyles involves the forward or backward travelling of the upper bone upon the lower, demanding a corresponding mobility of the intervening meniscus. The tendency for the surfaces to become parallel as a result of this movement, thus hindering lubrication within the joint, is usually counteracted by cambering of the lower articular facets, so that sliding of the menisci reduces the concavity of the whole receiving surface.

6. Ligaments help to restrict further movement as the close-packed position of the joint is neared. In most ligaments there are some taut fibres in all positions of the joint; these determine the relative proportion of rolling and gliding and, in addition, probably initiate a pattern of afferent nervous impulses, arising in the capsular and other ligaments, that is characteristic for each position.

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# THE OCULAR PARASYMPATHETIC NERVE SUPPLY AND ITS MESENCEPHALIC SOURCES

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It is usually stated that the parasympathetic innervation of the eyeball has its central origin in the Edinger-Westphal nucleus, and perhaps also in the other small-celled component of the oculomotor complex, the antero-median nucleus of Perlia (1889), and that their axons emerge from the midbrain in the third nerve, leaving it to relay in the ciliary ganglion, whence post-ganglionic fibres pass to the ciliaris and sphincter pupillae by the short ciliary nerves (Text-fig. 1). The literature concerning this pathway is vast, and only the more significant contributions can be mentioned here.

The post-ganglionic neurons were the earlier studied. Fallopius (1600) referred briefly to a plexiform junction between branches of the third and fifth nerves in the orbit. Willis (1664) first clearly described the short ciliary nerves; he wrote of a 'plexus rotundus', belonging to the oculomotor nerve, as their source. It was Schacher (1701) who recognized this as a ganglion, and its connexion with the trigeminal nerve in man was first depicted by Eustachius (1714), according to Meckel (1748). With the descriptions of Winslow (1732), and particularly Haller (1743), the arrangement of the short ciliary nerves, their ganglion, and its motor and sensory roots was clarified in man. Extensive comparative data were added by Zinn (1780), Muck (1815), Longet (1842), Budge (1855), Krause (1861), Schwalbe (1879), Jegorow (1886, 1887) and many others.

Ruysch (1722) and Winslow (1732) gave early accounts of the ramification of the ciliary nerves in the eyeball, and these have been amplified by later observers, especially Agababow (1893, 1912), Pines & Pinsky (1932), and Boeke (1933, 1936), who have reviewed the literature. Anatomical proof of innervation of the ciliaris and sphincter pupillae by these nerves was not early forthcoming, but Mayo (1823), Hall (1846), Bernard (1852) and Budge (1855) stimulated them electrically and produced meiosis; Muck (1815) and Longet (1842) cut them, and noted pupillo-dilation. Hensen & Völckers (1868) confirmed the pupillary response to stimulation and also recorded movements of the ciliary body. All these experimenters claimed that oculomotor stimulation caused meiosis, except Bernard (1852), whose disagreements focused interest on the nature of the ciliary ganglion, and of ganglia in general. Fallopius (1600), who introduced the term 'ganglion' to neurology, had regarded them as 'little brains', a view shared by Lancisi (1723), Winslow (1732), and Johnstone (1771).

The recognition, in man, of connexions between the ciliary ganglion and both the third and fifth cranial nerves intensified controversy over its function. Valentin (1839), Budge (1855), and Reichardt (1875) considered it a dependent of the third nerve, a view confirmed in all classes of vertebrates by Schneider (1879), Schwalbe (1879), Peschel (1893), Apolant (1896*a*) and Pitzorno (1913*a, b*). Schwalbe found no trigeminal connexion in teleosts, amphibians, or reptiles, but observed an oculomotor root in all mammals examined, as did Zeglinski (1885), d'Erchia (1894), and



Carpenter (1911) in birds. In spite of this, and of Arnold's (1831) previous suggestion that the ciliary ganglion was autonomic, most of these authorities thought it the homologue of a spinal root ganglion. Numerous and conflicting studies of its cells were reported. Reichardt (1875), Schwalbe (1879), Goldberg (1891), Peschel (1893), and Van Gehuchten (1893) considered them 'spinal', while Retzius (1881, 1884), Michel (1894), Apolant (1896*b*), von Kölliker (1896) and Onodi (1901) considered them 'sympathetic'. Holtzmann (1896) described them as sensory in amphibians but autonomic in birds and mammals. Krause (1881), Bernheimer (1897*a*), Bach (1899), Bumm (1899), Fritz (1899) and Marina (1901) found varying proportions of motor and sensory cells in the ganglion, according to a dual function.

Meanwhile, Gaskell (1885) had shown that 'Remak's' fibres in the oculomotor nerve reached the ciliary ganglion; Langley & Dickinson (1889) had introduced the nicotine block, which was soon applied to the ciliary ganglion by Langley & Anderson (1892). These classical studies clearly established the existence of an autonomic pathway to the eye by way of the third nerve and its ganglion. Anatomical experimentation confirmed Langley's work. Apolant (1896*a*), Bumm (1901) and Marina (1899) traced small degenerating fibres as far as the ciliary ganglion from the oculomotor trunk following its division. Schwalbe (1879), Bach (1896, 1899), van Biervliet (1899), and Marina (1899) also noted retrograde degeneration of most of the ganglion's cells after procedures such as ciliary neurectomy, removal of the eyeball or its contents.

Nevertheless, Marinesco, Parhon & Goldstein (1908), Müller & Dahl (1910), and Sala (1910) added to the complexity of cell types already described in the ciliary ganglion, and Pines & Friedmann (1927, 1929) claimed to recognize no less than eight categories in human and simian ganglia. Kiss (1932), Hollinshead & Clark (1935), and other workers, have subsequently differentiated less numerous types. All were agreed that small nerve cells, autonomic in character, formed the predominant type. Embryological observers, however, such as Carpenter (1906), Ganfini (1911), and Kuntz (1920), have closely associated the ciliary ganglion with the trigeminal as well as the oculomotor nerve in its development.

In contrast with these inconclusive histological investigations, animal experiments have largely corroborated the views of Gaskell (1889) and Langley & Anderson (1892). Hensen & Völckers (1868, 1878) had already produced meiosis and ciliary contraction by stimulation of the oculomotor trunk, ciliary ganglion, and short ciliary nerves in dogs, cats and monkeys. Their observations were repeated by Jegorow (1886), Spallita & Consiglio (1893), Langendorff (1894), Jendrassik (1896), Marina (1899), and François-Franck (1904), most of whom, like Angelucci (1899), Lodato (1900), and Anderson (1905), noted that stimulation failed to produce intra-ocular movements after division of the third nerve or destruction of the ganglion. Luco & Savvestrini (1942) and Kuntz, Richins & Casey (1946) have confirmed these findings.

It is apparent that much evidence, anatomical, physiological and experimental, has combined to prove that the sphincter of the iris and the ciliaris muscle are supplied by an autonomic pathway in the oculomotor and short ciliary nerves, which relays in the ciliary ganglion. On the other hand, proof of the precise source of the pre-ganglionic fibres of this pathway is much less satisfactory.

Although Spitzka (1888) claimed priority, Edinger (1885) and Westphal (1887)

are usually credited with the discovery of the paired groups of small cells, dorso-medial to the main oculomotor nucleus of man, which still bears their names. Perlia (1889) confirmed these observations, describing in addition another median mass of similar small nerve cells, the antero-median nucleus, situated at the cephalic end of the oculomotor complex. Panegrossi (1898) noted the latter in monkeys, and Siemerling (1891), Cassirer & Schiff (1894), Tsuchida (1906), Zweig (1921), Benjamin (1939) and Crosby & Woodbourne (1943), regarded these small-celled nuclei as a continuous mass, the antero-median nucleus forming a cephalic extension of the Edinger-Westphal columns.

The midbrain stimulation experiments of Hensen & Völckers (1868, 1878) and Adamük (1870) had indicated that cells near the cephalic end of the oculomotor nucleus innervated the ciliaris and sphincter, a view supported by the clinical arguments of Kahler & Pick (1881) and Starr (1888). It was therefore logical to suppose that the newly discovered Edinger-Westphal nuclei might be these centres. Clinical evidence in support of this was reported by Déjérine & Darkschewitsch (1887), Oppenheim (1888), Spitzka (1888), Knies (1891), Kostenitsch (1893), Jakob (1894), Pacetti (1894), Stuelp (1895), Pineles (1896), Ahlström (1900), Majano (1903), and Angelucci (1910). Some of these accounts were perhaps unduly dogmatic; Knies, for example, devised an elaborate scheme of oculomotor functions, on unspecified evidence, in which the Edinger-Westphal nucleus mediated accommodation, the pupillo-constrictor centre being Darkschewitsch's nucleus. Zeri (1895), von Bechterew (1897), Juliusberger & Kaplan (1899), and Bach (1906) disagreed with these views, which were indeed no more than clinical deductions from scanty pathological data. More extensive clinicopathological investigations by Siemerling (1891), Boedeker (1892), Cassirer & Schiff (1894), von Kölliker (1896), Siemerling & Boedeker (1897), Panegrossi (1898), von Monakow (1895, 1905), and Tsuchida (1906), produced no positive findings, although Brouwer (1918), Frank (1921), Grünstein & Georgieff (1925), and Lenz (1928, 1929) have more recently found pathological reasons to favour the Edinger-Westphal nuclei as the source of the ocular parasympathetic. Levinsohn (1917), Brouwer (1918), and Latumeten (1924) have reviewed *in extenso* this aspect of the literature.

Meanwhile, anatomical experimenters were likewise reporting contradictory results. Midbrain retrograde changes due to third nerve interruption and ciliary ganglionectomy were studied by Bernheimer (1897*a, b*), Bach (1899, 1900), van Biervliet (1899), Marina (1899), and Levinsohn (1904), of whom only Bernheimer and Levinsohn reported implication of the Edinger-Westphal nucleus, destruction of which, according to the former (1901), paralysed the sphincter pupillae. Van Gehuchten & van Biervliet (1901) merely admitted that the parasympathetic centre was probably cephalic in position in the oculomotor complex. Latumeten (1924) emphatically denied that Edinger-Westphal axons entered the third nerve, a view based on late midbrain changes in four cats on which Magnus had carried out two oculomotor divisions and two ciliary ganglionectomies. Crouch (1936), in a more extensive series of cats, found no clear response to ganglionectomy, but the results of third nerve injury were clear enough to lead him to conclude that some of the Edinger-Westphal fibres crossed before entering it. Kuré, Susuki, Kaneko & Okinaka (1933) found ganglionectomy regularly effective in dogs.

Comparative methods have also led to conflicting conclusions. Panegrossi (1904), Tsuchida (1906), Ramon y Cajal (1911), and Neiding & Frankfurter (1911) discounted the Edinger-Westphal column as a radicular oculomotor nucleus, but Brouwer (1918) claimed that it mediated accommodation; he compared its progressive differentiation in mammals with the development of Perlia's nucleus (convergence), regarding both as closely associated with the evolution of binocular vision. Zweig (1921), on morphological grounds alone, was prepared to assign the functions of pupillary constriction and accommodation to the Edinger-Westphal and antero-median nuclei respectively. Le Gros Clark (1926) concluded that comparative data merely suggested the inclusion of these nuclei in the oculomotor complex, and subsequent topographers of these centres, such as Crosby & Woodburne (1943), have likewise expressed reserved opinions.

Little pertinent embryological information exists. Cramer (1894) and Tsuchida (1906) could not distinguish the Edinger-Westphal nucleus before the 7th month. Magitot (1921) has observed an active light reflex early in the 6th month; but this is not a serious discrepancy, since Hertel (1907) has shown that the human iris may respond directly to light. Moreover, Paton & Mann (1925) and Mann (1927) identified the nucleus in 48 mm. embryos, and Pearson (1944) at the 5th month, at which stage its cells are already distinguishable from somatic oculomotor neurones, according to Malone (1913). Cooper (1946) identified the nucleus even earlier, at the 40 mm. stage.

Recent experimenters have returned to stimulation methods, with the modern advantages of stereotaxic instruments. Ranson & Magoun (1933) found that stimulation in or near the Edinger-Westphal nucleus caused ipsilateral meiosis. Benjamin (1939) agreed with this but included the antero-median nucleus, whereas Szentágothai (1943) thought the Edinger-Westphal alone was concerned. All these workers used cats. In monkeys Bender & Weinstein (1943) could not accurately locate centres for meiosis or accommodation.

From this review it is apparent that the origin of the ocular post-ganglionic fibres from the ciliary ganglion has been established. That the pre-ganglionic fibres issue in the oculomotor nerve to relay in the ganglion is not so well authenticated. The precise central source of these fibres remains in doubt. All methods of study have led to disagreements. It was therefore decided to re-examine the problem, with special regard to the location of the pre-ganglionic nerve cells.

#### MATERIALS AND METHODS

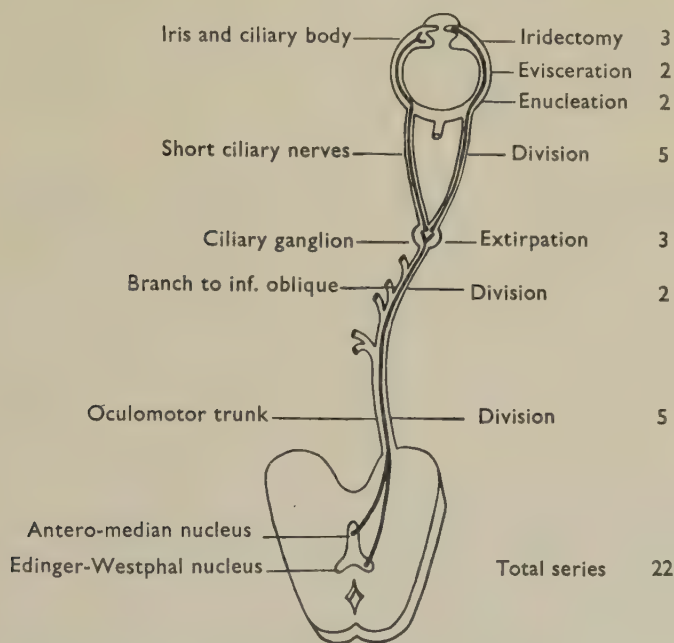
The observations of this research were made upon the midbrains and ciliary ganglia of twenty-three monkeys (*Macaca mulatta*) and five cats. The monkeys were submitted to the following operative lesions (Text-fig. 1):

(1) Iridectomy	3
(2) Exenteration of the eyeball	2
(3) Removal of the eyeball	2
(4) Division of the short ciliary nerves	5
(5) Extirpation of the ciliary ganglion	3
(6) Division of the nerve to the obliquus inferior	2
(7) Division of the inferior oculomotor ramus	1
(8) Division of the oculomotor trunk	5



The cats were all subjected to procedure 4. In addition to this material, serial sections prepared from the midbrains of a large number of monkeys were also available for examination of topographical details.

All the operations were unilateral and were performed under intravenous or intraperitoneal anaesthesia (Kemithal, Nembutal, or Pentothal). The ocular operations were carried out in the usual manner and presented no special difficulties. Interruption of the short ciliary nerves was effected through a wide incision in the



Text-fig. 1. This diagram illustrates (with the exception of the inclusion of the antero-median nucleus) the anatomical pathway from the midbrain to the eyeball as usually described in text-books. On the right of the diagram are listed the procedures carried out in this research. The figure indicate the number of monkeys used in each kind of experiment.

upper lid; careful dissection within the muscle cone led to identification of the optic nerve, around which the short ciliary branches were isolated and divided. The ciliary ganglion was usually approached through the lower lid. This route permitted early recognition of the inferior oblique and its nerve supply. The nerve was followed back under the eye towards the apex of the orbit by gentle dissection, chiefly carried out by means of pledgets of cotton-wool soaked in 1/1000 adrenalin hydrochloride solution. This method kept the operation field dry, an important point in searching for such small structures in so restricted a working space. It was usually possible to find the ganglion, but to isolate it clearly enough to sever its connexions under direct vision was difficult and sometimes impossible. In the latter eventuality the short ciliary nerves were divided instead, since blind cutting under such conditions may easily divide other structures than those intended. The short ciliary nerves were always cut well anterior to the ganglion, because of the danger of interfering

inadvertently with its blood supply, which is said to reach it by branches from the muscular and posterior ciliary branches of the ophthalmic artery. It was clear, however, that the effects produced in the ciliary ganglion by this procedure were not an artefact due to devascularization, since precisely the same results followed removal of the ocular contents.

Exposure of the ganglion by removal of the lateral orbital wall was also tried, but this afforded even less space. To ensure that ganglionectomy had been accomplished, the excised nervous tissue was always sectioned; post-mortem dissection of both orbits was also always carried out, not only as a further check upon the effectiveness of the operation, but also to secure the normal ganglion from the undisturbed orbit for comparison. Although these procedures were sometimes lengthy, all animals made rapid and uneventful recoveries. No post-operative infections occurred.

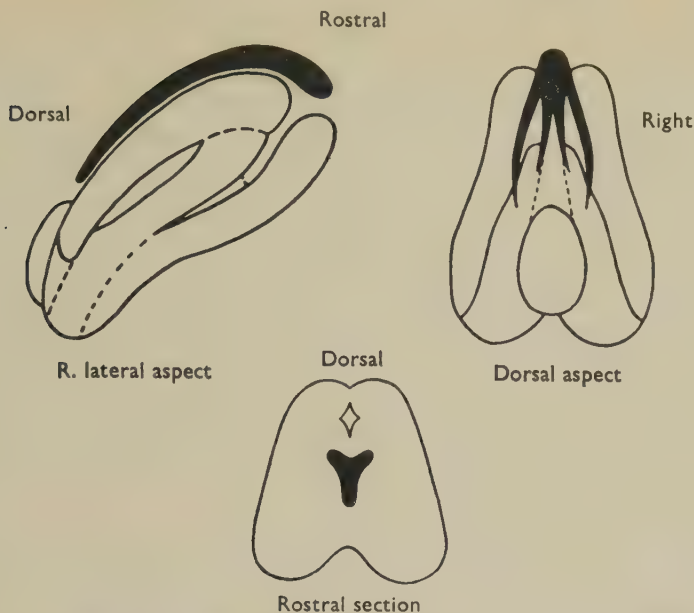
At periods varying from 8 to 16 days after operation each animal was anaesthetized as above, bled, and perfused with 10% formol saline solution at a pressure maintained at 120 mm. of mercury by a small pump. The midbrains and ganglia were carried through to paraffin wax embedding in the usual manner. All were sectioned serially at  $10\mu$  thickness. Sections were stained by Bielschowsky's dilute cresyl fast violet technique in most cases, some by Einarson's gallocyenin method.

## RESULTS

### *Topography*

A detailed description of the topography of the oculomotor complex, including its parasympathetic nuclei, will form part of a later communication, but a brief note of the arrangement of these centres is necessary as a preface to the experimental results which follow.

The Edinger-Westphal nucleus of each side consists of a slender column of small multipolar cells scattered irregularly and in small numbers in sections through the cephalic three-fifths of the main third nerve nucleus, to which they are dorsal at caudal levels, becoming dorso-medial in position at more cephalic levels (Text-fig. 2). Thus the right and left columns approach each other, fusing across the midline raphé at the cephalic extremity of the oculomotor complex (Pl. 1, fig. 2). A ventral extension of this conjoined mass, of similar nerve cells, arches over the cephalic aspect of the main oculomotor mass in the midline, forming the so-called antero-median nucleus (nucleus medianus anterior of Perlia) (Pl. 1, fig. 1). Dorso-lateral to the cephalic half of the Edinger-Westphal columns an ill-defined group of like cells can sometimes be made out in the monkey; this, the lateral Edinger-Westphal nucleus of human topography, merges with the main or medial Edinger-Westphal nucleus caudal to the fusion of the latter with its fellow. Although regional names were applied to parts of this mass of small motor neurones before their confluence was clearly recognized, it is important to note that their continuity is complete in the monkey (Text-fig. 2). Throughout this account, however, the established usage of the terms 'Edinger-Westphal' and 'antero-median' nuclei has been followed, although it is apparent that the latter is a cephalic prolongation of the Edinger-Westphal columns.



Text-fig. 2. These diagrams represent the topographical structure of the oculomotor complex in the rhesus monkey. The parasympathetic nuclei (in black) are the Edinger-Westphal columns, dorsal to the somatic nuclei, and the midline antero-median nucleus, formed by the coalescence of these columns at the cephalic or rostral extremity of the complex.

## EXPERIMENTAL RESULTS

### (a) *Division of the short ciliary nerves*

The bundles of short ciliary nerves were completely severed in one orbit of five monkeys and five cats. Serial sections of the right and left ciliary ganglia, and of the midbrain, were examined in each experiment. No changes were observed in the oculomotor or other mesencephalic nuclei of these animals, but in the ganglion from the side of operation extensive and unmistakable retrograde degeneration was always evident. The ganglion of the opposite side, which was never affected, provided a group of normal cells for comparison. The normal ciliary ganglion neurones, both in the monkey and the cat, possess cytons averaging respectively  $45 (30-63)\mu$  and  $35 (15-30)\mu$  in size. In preparations stained for chromatin material they appear multipolar and contain uniformly distributed Nissl granules, which are numerous, fine and sometimes elongated (Pl. 2, fig. 4). In the series inspected, most of the cells were alike, and no elaborate range of types could be identified. There was some variation in size, and in the amount of visible chromatin substance, but the only other distinct type of cell encountered was extremely infrequent; it was much smaller, containing a few large granules around a relatively large nucleus (Pl. 2, fig. 5). This type of cell averaged  $23 (15-30)\mu$  in size in the monkey; it was not satisfactorily identified in the cat's ganglion. Normal cells exhibiting chromophily were seen occasionally in some series, but were absent from most. Their significance, which has been discussed elsewhere (Warwick, 1951, 1953*b*), was clearly unrelated to the retrograde changes studied.



In the ganglion from the side of operation the classical phenomena of the retrograde reaction, chromatolysis and nuclear eccentricity, were clear in every series (Pl. 2, fig. 6). Extrusion of nuclei was seen less often, and the swelling characteristic of acute retrograde degeneration in some kinds of nerve cells was not evident in ganglia from monkeys, a slight degree of shrinkage being more usual (Pl. 2, figs. 6, 7). Swelling was present but never marked in the cats' ganglia; in both animals chromatolytic cells were sometimes rounded and sometimes misshapen. A chromophil stage in the reaction was occasionally encountered, and the peripheral clumping of chromatin material typical of the earlier phases of chromatolysis was seen in some cells (Pl. 2, fig. 7). As reported previously (Warwick, 1951, 1953*b*), in connexion with retrograde changes in somatic oculomotor neurones, the most striking and reliable feature was found to be chromatolysis. This was apparent to a marked degree in almost all the cells in each series, and in many all visible chromatin material had disappeared (Pl. 2, figs. 6, 7). Counts of normal and degenerating cells were made in numerous sample fields in four ganglia from the side of operation. These figures are tabulated herewith:

Animal	Sections examined	Total cells	Normal cells	Percentage chromatolytic
Cat no. 5	20	2690	104	96.1
Cat no. 6	20	2480	75	97.1
Monkey no. 87	20	2595	71	97.2
Monkey no. 88	23	3102	84	96.8

These counts show that an almost universal degeneration of the ciliary ganglion's cells followed division of its short ciliary branches. The widespread and unequivocal nature of the reaction was so evident that the normal and affected ganglia could be distinguished easily under magnification low enough to include them in the same field (Pl. 1, fig. 3).

(b) *Exenteration of the eyeball and iridectomy*

Evacuation of all tissues within the scleral tunic was carried out in two monkeys. In both the ipsilateral ciliary ganglion showed retrograde changes as marked and widespread as were those resulting from ciliary neurectomy. Normal cells were extremely few. This manoeuvre naturally interrupted all nerve fibres entering the eyeball, including those from the ciliary ganglion. To estimate the proportion of the cells in the ganglion whose fibres enter the iris, a unilateral iridectomy was performed in three other monkeys. In each case most of the cells in the ipsilateral ganglion were normal; but occasional neurones, scattered singly or in small groups, were plainly degenerating (Pl. 2, fig. 7). Counts of normal and chromatolytic cells were made in numerous sample fields in two of the affected ganglia; the results were as follows:

Series	Sections examined	Total cells	Chromatolytic cells	Percentage
83	20	3027	95	3
85	20	3687	135	3.5

Equal numbers of serial sections of the ganglia of the opposite side were also examined in all three animals; the cells in these were all normal.

*(c) Ciliary ganglionectomy*

Removal of the ciliary ganglion on one side was accomplished in three monkeys. Following this procedure clear retrograde changes were evident in the cells of the Edinger-Westphal and antero-median nuclei ipsilateral with respect to the orbital lesion. The normal cells of these groups were seen to be small and usually multipolar, ranging from 15 to 25  $\mu$ . By their size, their relatively large nuclei, and the paucity of their Nissl granules, they were easy to distinguish from the neighbouring somatic oculomotor nerve cells, and the comparatively undifferentiated cells of the surrounding central grey matter (Pl. 3, fig. 8). The cells of the antero-median nucleus appeared to be little different from those in the Edinger-Westphal columns, being merely somewhat elongated in the sagittal plane (Pl. 3, figs. 9, 11). The usual features of retrograde degeneration were visible in the cells of both nuclei, except for swelling, which was not always seen and rarely marked, although the cells were usually rounded (Pl. 3, fig. 10). On the other hand, a chromophil stage in chromatolysis was often observed (Pl. 3, fig. 12).

Degenerating cells were confined to the ipsilateral Edinger-Westphal nucleus and the ipsilateral half of the antero-median nucleus (Pl. 3, figs. 8, 9). In the latter the contrast between normal and affected neurones lying on opposite sides of the mid-sagittal plane was especially noticeable (Pl. 3, fig. 9). Inspection of serial sections through the whole length of the Edinger-Westphal columns showed that the proportion of cells affected was less here than in the antero-median nucleus, where chromatolysis was almost universal in the cells on the side of operation. Nevertheless, retrograde changes were seen in cells at all levels of the ipsilateral Edinger-Westphal group. No unequivocal degeneration was noted in any of the cells of the contralateral nuclei.

In one series some of the cells in the ipsilateral somatic oculomotor nucleus also showed chromatolysis. These were grouped in a manner corresponding to the results of resection of the obliquus inferior, as reported elsewhere (Warwick, 1953*b*). Post-mortem dissection of the orbit of this monkey showed that this muscle's nerve supply had been divided during ganglionectomy, thus accounting for these additional effects.

*(d) Orbital interruption of the oculomotor nerve*

Serial sections were examined from the midbrains of two monkeys submitted to deliberate division of the nerve to the obliquus inferior and one in which the inferior oculomotor ramus had been cut. In all three preparations the lesion, checked by post-mortem dissection, was proximal to the origin of the motor radix of the ciliary ganglion. In each series ipsilateral retrograde changes were observed in the Edinger-Westphal and antero-median nuclei. The results were in every way identical with those caused by ciliary ganglionectomy.

*(e) Intracranial division of the oculomotor nerve*

The oculomotor complex was inspected serially in the midbrain of five monkeys which had been subjected to unilateral division of the oculomotor trunk close to its superficial origin from the cerebral peduncle. In addition to changes in the main third nerve nuclei, which have already been described (Warwick, 1951, 1953*a, b*),

retrograde degeneration was marked in the ipsilateral Edinger-Westphal and antero-medial nuclei. The cells of these were never affected by divisions of the branches of the third nerve, except when the nerve to the obliquus inferior was cut proximal to its ciliary branch. By exclusion, therefore, these small-celled nuclei of the oculomotor complex cannot be concerned with the innervation of the extra-ocular muscles, but must supply the fibres which relay in the ciliary ganglion to supply the eyeball.

#### DISCUSSION

Concerning the post-ganglionic neurones of the ocular parasympathetic supply agreement may be said to have been reached, except for minor details; the voluminous literature about them therefore requires no protracted discussion. Bernheimer (1897*b*, 1898), Fritz (1899), Marina (1899, 1901), and Marinesco, Parhon & Goldstein (1908) claimed that some of the cells of the ciliary ganglion innervate the cornea. Although most workers who have examined these cells have deemed them motor in function, the occurrence of sensory cells in this ganglion is a possibility which cannot be entirely refuted. The almost uniform nature of the cell population in all the ganglia examined in this investigation makes it unlikely that any are sensory. It is more probable that all the cytons of corneal afferents lie in the trigeminal ganglion, changes in some of the cells of which follow corneal injury, as Marina (1899) has shown.

Langendorff (1894), Moeli (1897), and François-Franck (1904) suggested that the ciliary ganglion might be a peripheral reflex centre, and Clark (1937) thought that it contained internuncial neurones, despite his own observation that total degeneration of its cells followed division of the short ciliary nerves. It could be supposed that such internuncial cells, if they existed, might exhibit transneuronal degeneration, but in view of our ignorance of the connexions they might effect, such considerations are purely hypothetical. All such views of the existence of different functional types of neurone in this ganglion are seriously weakened by electrophysiological studies of its cells by Whitteridge (1937). He found that in the monkey they all behaved alike, and he could not support concepts of the ganglion as a co-ordinating centre.

Kuré, Susuki, Kaneko & Okinaka (1933) stated that dystrophic changes occurred in the extra-ocular muscles after destruction of the ciliary ganglion. It is not clear how the axons of such cells were thought to reach the muscles. This view has received no corroboration, and the present findings contradict it, since almost all the ganglion's cells reacted to exenteration of the eyeball, a procedure which could not disturb such supposed arrangements.

The association of the ciliary ganglia with the third nerve is no longer in doubt, and the fact that the nerve contains motor fibres for the sphincter pupillae and ciliaris is attested by an imposing accumulation of evidence. Numerous workers, over a period of almost a century, have cut and stimulated the oculomotor nerve, noting, with few exceptions, some sort of pupillary response. It is true that some disagreement has arisen over the precise mechanisms of pupil movements. Von Bechterew (1883) first suggested that dilatation in response to pain was due to central parasympathetic inhibition rather than to sympathetic activation of the dilator muscle. Braunstein (1894) confirmed this idea, while Karplus & Kreidl (1912, 1918) admitted that reflex mydriasis was not wholly effected by sympathetic



activity in the iris. The participation of parasympathetic inhibition in reflex pupil dilatation resulting from other stimuli, including light withdrawal, has been confirmed by McDowall (1925), Byrne (1933), Bain, Irving & McSwiney (1935), Gullberg, Olmsted & Wagman (1938), Urey & Gellhorn (1939), Hodes (1940), and others. Kuntz & Richins (1946), like some previous workers, found that division of the third nerve abolished reflex pupil dilatation, as did also ciliary ganglionectomy in their experiments. These views are in accord with the results of cortical stimulation. Pupillary dilatation in response to this was first noted by Bochart (1875), and Parsons (1901) reviewed the early literature, noting that the dilatation was abolished in dogs and cats by oculomotor division. Karplus & Kreidl (1910), Ingram, Ranson & Hannett (1931), Harrison, Magoun & Ranson (1938), Urey & Oldberg (1940), Hodes & Magoun (1942*a, b*), and Ward & Reed (1946) adduced evidence that an inhibitor pathway, of extra-pyramidal nature, descends from the vicinity of the frontal and occipital eye-fields via the hypothalamus to the tegmentum. They considered that excitation of this produced pupil dilatation by inhibition of oculomotor nerve cells. Keller (1946) has claimed that the cells of the Edinger-Westphal nuclei continue to discharge after isolation of the oculomotor centres by brainstem transections, an activity which, as Adler (1950) has suggested, would support the concept of central parasympathetic inhibition in dilatation of the pupil. Whether such interpretations prove correct or not, the experiments which formed their basis have uniformly confirmed the innervation of the sphincter pupillae by way of oculomotor fibres.

In a similar manner more complex ideas of the nervous control of accommodation have been advanced. Helmholtz (1855) first suggested a duality of function in the ciliaris, a concept also sponsored by Henke (1860). Morat & Doyon (1891) thought that the ciliaris received sympathetic and parasympathetic nerve supplies, mediating respectively distant and proximate accommodation. Jessop (1886) produced flattening of the lens by stimulation of the cervical sympathetic, and a controversial literature, reviewed by Cogan (1937), has since accumulated. Latterly, Jessop's claims have received fresh support from the experiments of Morgan, Olmsted & Watrous (1940), Olmsted & Morgan (1941), Mohoney, Olmsted, Morgan & Wagman (1942), and Olmsted (1944). Clark (1937) and Kuntz & Richins (1946) did not favour these views, and Stotler (1937) reported complete denervation of the ciliaris by ciliary ganglionectomy in the cat, an animal in which, according to Jegorow (1886), Anderson (1905), and Christensen (1934, 1936), no sympathetic fibres traverse the ganglia. Although a dual innervation of the ciliaris thus remains uncertain, it is noteworthy that none of these workers found reasons to doubt that it is supplied by oculomotor axons.

Certain observations concerning regeneration of the human third nerve after peripheral injury may be alluded to here. Bender & Alpert (1937), Ford, Walsh & King (1941), Bender (1945), Cristini (1947), and Russell & Wright (1948) have described the abnormal synkineses which characterize recovery of ocular movement in such cases. Bender & Fulton (1939) have noted like phenomena in the chimpanzee and monkey. It was commonly found by these authorities that abnormal nerve supplies were established. They ascribed this to mis-shunting, and it is of interest here to note that the sphincter pupillae, and sometimes the ciliaris,

were involved in these effects, a further indication of the innervation of these muscles by way of the third nerve.

In contrast to this mass of evidence, confirming the existence of an ocular parasympathetic element in the third nerve, uncertainty persists with respect to the precise central origin of these autonomic pre-ganglionic fibres. Clinico-pathological deductions, prominent in the earlier literature, produced contradictory views. It is well recognized that chronic morbid changes in brain stem nuclei are not easy to assess, and the more so in the case of small-celled and inconspicuous nuclei, such as these under discussion. The negative findings of even the more extensive investigations regarding the Edinger-Westphal nucleus are therefore unconvincing.

Most evidence obtained by experimental anatomy has been against the Edinger-Westphal nuclei as oculomotor parasympathetic components. Bernheimer (1897*b*) was almost unique among contemporary experimenters in stating that they were the source of the ocular parasympathetic. He regarded these nuclei as pupillo-constrictor centres and Perlia's nucleus (despite its somatic type of nerve cells) as the centre for the ciliaris. His conceptions of general oculomotor function have been shown to be largely inaccurate (Warwick, 1953*b*). His claims that enucleation and other lesions distal to the ciliary ganglion produced retrograde changes in the mid-brain provoked criticism by his contemporaries, such as Bach (1899), who were aware of the convincing proof of a relay in the ganglion reported by Langley & Anderson (1892). These errors detract from the credibility of Bernheimer's results, which nevertheless were accepted almost in their entirety by Brouwer (1918). If the axons of nerve cells in the Edinger-Westphal group do indeed issue in the third nerve, cutting it should cause changes in these cells. The evidence of this research was entirely positive on this point and to this extent confirmed the findings of Bernheimer (1897*a*), Levinsohn (1904), and Crouch (1936). Latumeten (1924), whose opinions have attracted perhaps disproportionate interest, reached a negative conclusion, based upon experiments on two cats. (It is noteworthy that Crouch's work involved nine.) For technical reasons, detailed elsewhere (Warwick, 1951, 1953*b*), little weight can be attached to Latumeten's emphatic denial that Edinger-Westphal fibres enter the third nerve.

Physiological evidence, on the contrary, has clearly and almost uniformly indicated a pupillo-constrictor and accommodation pathway, of autonomic type, in the oculomotor nerve. Midbrain stimulation experiments, from the classical studies of Hensen & Völckers (1868, 1878) to those of Ranson & Magoun (1933) and Szentágothai (1943), have provided less agreement concerning the nuclei of origin of these fibres; but most of those who have used such methods have found indications of a centre for the sphincter, and sometimes one for the ciliaris, both near, if not identical with, the Edinger-Westphal nuclei. It has been objected that such methods, even with modern refinements, are not exact enough to identify such small cell masses, even if supplemented, as in Szentágothai's work, by tracing degenerating fibres into peripheral nerves from central destructive lesions. Nevertheless, the existence of a pupillo-constrictor centre, at the cephalic end of the oculomotor nucleus, appears to have been amply established by such means.

Although the histological effects of oculomotor division have demonstrated more certainly than physiological methods that some of the oculomotor axons come from

the Edinger-Westphal nuclei, their function can only be deduced from such findings. If all other radicular neurons of the third nerve can be made to show retrograde changes by division of its muscular branches, it is justifiable to presume that these small-celled nuclei are the source of the fibres which relay in the ciliary ganglion. Such was Bernheimer's view (1897*a*). Studies reported previously (Warwick, 1951, 1953*b*) have demonstrated that only the antero-median and Edinger-Westphal nuclei, among all the nerve cells affected by oculomotor trunk division, remain unaltered by lesions of the branches of the third nerve, provided that the injury is distal to the motor root of the ciliary ganglion in the case of the nerve of the obliquus inferior. This permits a strong presumption that the axons which issue from these nuclei pass from the third nerve into the ciliary ganglion, as commonly accepted. As stated above, the retrograde effects of short ciliary nerve injury have often been demonstrated, and this was confirmed in both the cat and the monkey in this research. Ciliary ganglionectomy is therefore the crucial anatomical experiment in linking up the ocular post-ganglionic fibres with the midbrain source of the pre-ganglionic axons of this pathway. Levinsohn (1904), from such procedures in nine cats, concluded that these fibres originate in the Edinger-Westphal nuclei; Bach (1906) decided that in rabbits they do not. Both authors inspected each other's preparations and disagreed with their respective interpretations: (Bach used the Weigert technique, unsuitable for observations on chromatin granules). Latumeten (1924) and Crouch (1936) also recorded negative findings, in two and nine cats respectively, but Kuré, Susuki, Kaneko & Okinaka (1933) described retrograde changes as constant in the Edinger-Westphal nuclei after ciliary ganglionectomy. No previous workers appear to have described such experiments in monkeys. My results in this animal were unequivocal; in each experiment both the Edinger-Westphal and antero-median nuclei were the seat of widespread chromatolysis. Only Levinsohn (1904) has also included the antero-median nucleus in the changes produced by ganglionectomy. This nucleus has rarely been mentioned by experimental anatomists, although the changes noted in it in this investigation were particularly striking.

Certain other views may be conveniently considered here. Von Bechterew (1883) and Mendel (1887) suggested the habenula nucleus (ganglion habenulae) as the pupillo-constrictor centre, but this view has not been corroborated by others. Darkschewitsch (1889) thought that his nucleus might perform this role; evidence excluding this nucleus from the oculomotor complex has been recorded by Ingram & Ranson (1935) and Warwick (1953*a*). Von Bechterew (1897), von Monakow (1895, 1905), Tsuchida (1906), and Mingazzini (1913, 1928) believed that the sphincter was controlled by cells scattered in the central grey matter near the floor of the third ventricle and aqueduct. These views, and Frank's conception of the Edinger-Westphal nucleus as a convergence centre, are contradicted by the present findings. Bernheimer's opinion that the central nucleus of Perlia innervated the intrinsic ocular muscles, ousted by Brouwer's ideas, was revived by Foerster, Gagel & Mahoney (1936); but, while Bernheimer had included the Edinger-Westphal nucleus as a part of this centre, they could find no changes in it after oculomotor divisions in ten monkeys and one chimpanzee. (They claimed that ciliary ganglionectomy confirmed this result, but gave no details of this aspect of their work.) Evidence that Perlia's nucleus, which is rarely a distinguishable entity in monkeys,



consists of nerve cells supplying extrinsic rather than intrinsic musculature, has been reported (Warwick, 1951, 1953*b*). It is improbable that the large motor cells, forming the central nucleus pictured by these authors, could be the cytons of autonomic neurones, although accommodation is a function which, by virtue of its close association with the conscious use of the eyes, might be regarded as voluntary in nature. It is perhaps curious that such an activity appears to be carried into effect by an autonomic pathway, which is also the efferent limb of the light reflex arc. This nervous pathway is itself unusual in the myelination of its post-ganglionic axons, a peculiarity indicated by Gaskell (1885) and coupled by him with the striated condition of the avian ciliaris. Lenhossék (1911) has drawn attention to the large size of these axons and of the ciliary ganglion cells in birds. Nevertheless, the ciliaris is non-striated in lower vertebrates and remains so in mammals, a condition consonant with its type of innervation. It may be suggested that as the mesencephalic reflex mechanisms of the visual function have become progressively replaced by an increasing degree of cortical control, this process of encephalization has affected not only the control of accommodation but also of the pupil in the reaction of convergence. This might explain not only the preservation of an autonomic pattern of innervation for the ciliaris and sphincter pupillae, but also the continued close anatomical association of the neurones supplying them.

Separate midbrain centres have sometimes been ascribed to these two muscles, but the uncertain pathological evidence upon which such hypotheses have been built makes their validity highly dubious. Stimulation experiments have failed to reveal such separate centres, and since trans-neuronal degeneration does not occur at the ciliary ganglion, it seems improbable that anatomical methods could do so. A comparison of the results of iridectomy and exenteration of the eyeball has shown that most of the ciliary ganglion's cells innervate the ciliaris and that few of their axons enter the iris. The bulk of the ciliaris relative to the sphincter leads one to expect that this would be so. It is logical to assume that the greater number of the fibres of the conjoined mass of the antero-median and Edinger-Westphal nuclei therefore innervate the ciliaris. Although the cells of the Edinger-Westphal columns appear in sections through more than the cephalic half of the oculomotor complex, they are more numerous at cephalic levels, where the columns coalesce to become continuous with the antero-median nucleus. The parasympathetic component of the oculomotor centres thus occupies a predominantly cephalic position, a finding which accords with the results of most stimulation studies and with the views of many clinical observers.

The antero-median nucleus, the most cephalic portion of the parasympathetic group of the third nerve complex, has attracted little attention, even as a topographical entity. Its continuity with the Edinger-Westphal nuclei, although not yet a feature of text-book accounts, has been confirmed in this research, the experimental results of which have also shown that both groups of cells have the same autonomic function. Szentágothai (1943), from an extensive stimulation study in cats, discounted the antero-median nucleus as a source of ocular parasympathetic fibres, a conclusion refuted by the present results, which confirm in this regard the experiments of Levinsohn (1904) and Benjamin (1939). Neither used monkeys. Crouch (1936) considered that some of the Edinger-Westphal axons decussated

before entering the third nerve; as in Benjamin's stimulation experiments, my experience was that they do not. Similarly, the fibres derived from the antero-medial nucleus, a midline structure, were observed to issue in the oculomotor nerve on the side of their own half of the nucleus.

It must be concluded that the usually accepted conception of the ocular parasympathetic pathway is correct, both as regards its peripheral route and central origin. It may be added that the pre-ganglionic fibres do not decussate in monkeys, and are derived not only from the Edinger-Westphal columns, as usually described, but also from their infrequently included cephalic extension, the antero-medial nucleus.

#### SUMMARY

The extensive literature concerning the ocular parasympathetic nerve supply is reviewed. Although convincing evidence exists that this pathway issues in the oculomotor nerve and relays in the ciliary ganglion, its mesencephalic sources, reputedly the Edinger-Westphal nuclei, are less satisfactorily substantiated.

The retrograde response to interruption at numerous points in this pathway was therefore studied in cats and particularly in monkeys; the latter have seldom been used in investigating this problem.

All lesions affecting the short ciliary nerves (e.g. enucleation, exenteration, ciliary neurectomy) produced chromatolysis in about 97 % of the cells of the ciliary ganglion. After iridectomy about 3 % only of these cells showed such changes.

These results confirmed that practically all the cells in the ciliary ganglion innervate intrinsic ocular musculature; they also showed that a small fraction only of their axons supply the sphincter pupillae.

Ciliary ganglionectomy and division of the third nerve (in monkeys) regularly caused a retrograde reaction of striking degree in most of the cells of the ipsilateral Edinger-Westphal nucleus and in the ipsilateral half of the antero-medial nucleus. Topographical observation showed that these nuclei form a continuous small-celled mass in the macaque.

It must be concluded that the usual account of the Edinger-Westphal nucleus as the parasympathetic component of the oculomotor complex is correct, and that the antero-medial nucleus is an integral part of this centre.

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## EXPLANATION OF PLATES

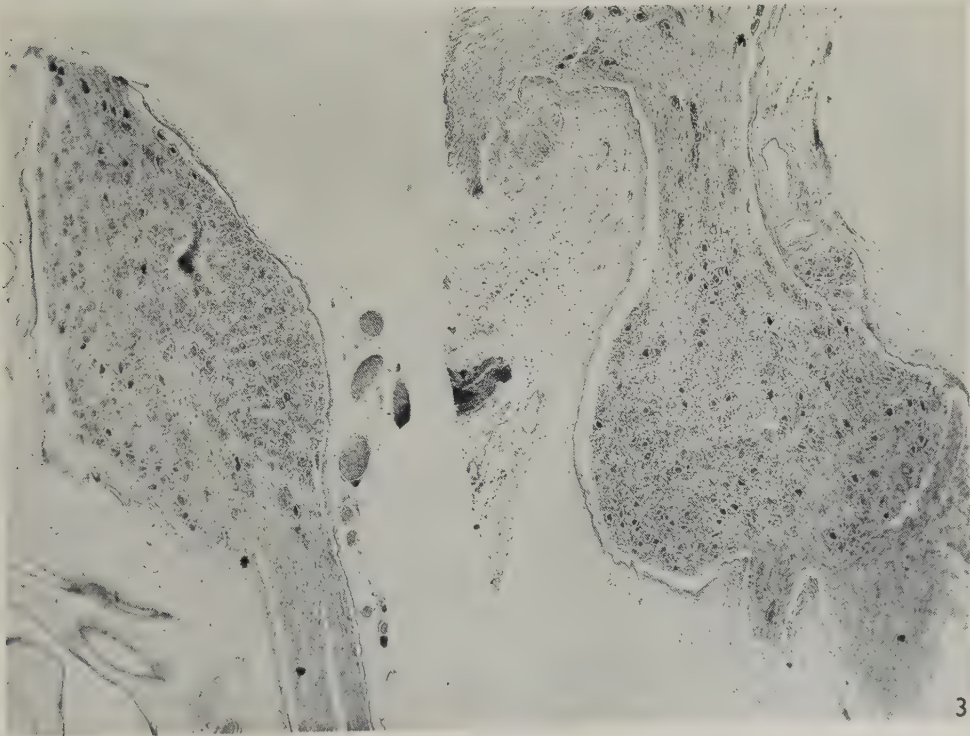
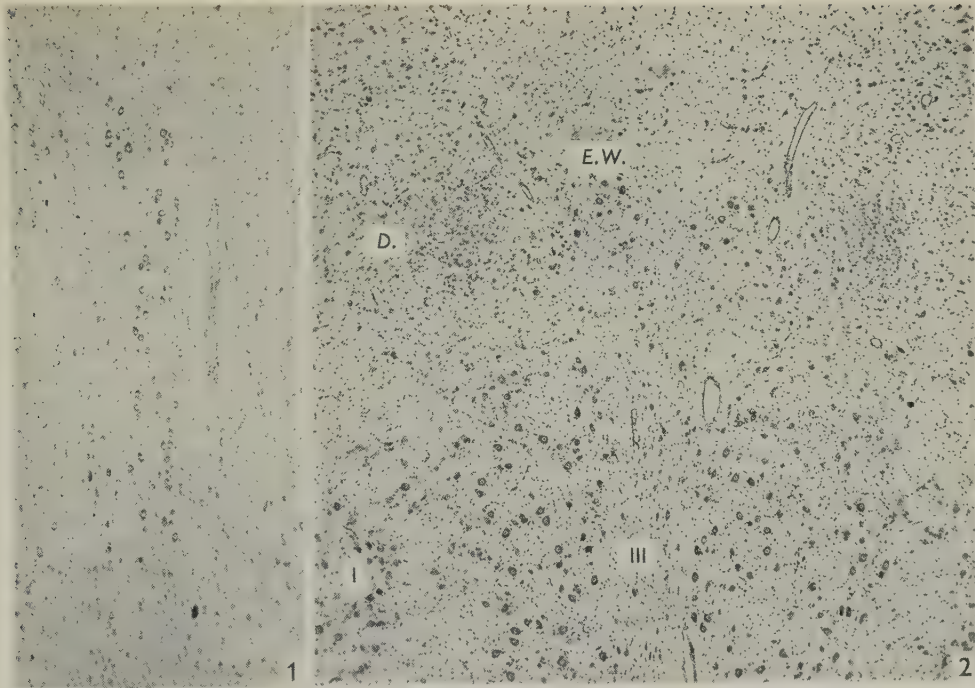
## PLATE 1

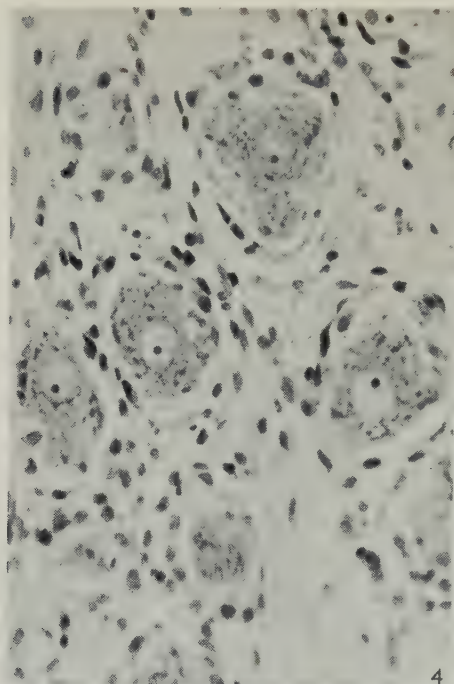
- Fig. 1. A transverse section just rostral to the main oculomotor nuclei. The Edinger-Westphal columns have fused caudal to this and are extended ventro-dorsally in the midline as the antero-median nucleus. Cresyl fast violet.  $\times 32$ .
- Fig. 2. This field shows the various cell groups visible in transverse sections through the rostral third of the oculomotor complex. Note the Edinger-Westphal columns (E.W.), which lie dorsal to the main third nerve nuclei (III) and are approaching close to each other at this level. The interstitial nucleus (I) and the nucleus of Darkschewitsch (D) are also shown. Cresyl fast violet.  $\times 32$ .
- Fig. 3. Sections through the left and right ciliary ganglion of a monkey subjected, 11 days before death, to removal of the contents of the right eye. Even at this low magnification it is obvious that most of the cells in the right ganglion (on the right in the photograph) are paler than those in the normal left ganglion of this animal (on the left). The pallor is due to chromatolysis. Cresyl fast violet.  $\times 50$ .

## PLATE 2

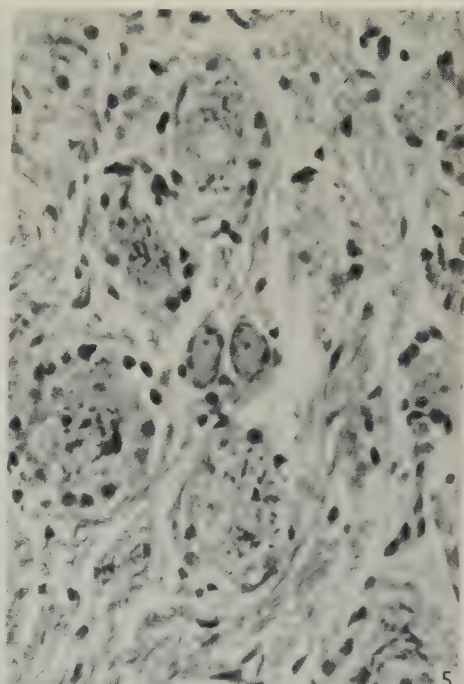
- Fig. 4. Nerve cells in a normal ciliary ganglion from a rhesus monkey. Note the prominent and evenly distributed chromatin granules and central nuclei of these neurones. Cresyl fast violet.  $\times 176$ .
- Fig. 5. Another field in a normal ciliary ganglion to show the only other type of cell encountered in this ganglion in the present research. Two such cells appear amongst the usual type; note their small size and peripheral disposition of chromatin granules. Cresyl fast violet.  $\times 176$ .
- Fig. 6. Degenerating nerve cells in the right ciliary ganglion of a monkey killed 10 days after division of the short ciliary nerves in the right orbit. All the cells show pronounced retrograde reaction, in their loss of Nissl granules and nuclear eccentricity. The dense appearance of one cell exemplifies the chromophil phase of chromatolysis seen in many types of neurone during retrograde degeneration. Contrast these cells with those in the left ciliary ganglion of the same animal (fig. 4). Cresyl fast violet.  $\times 176$ .
- Fig. 7. Field in ciliary ganglion showing chromatolysis in a small group at its nerve cells following iridectomy on the same side. Many of the cells contain obvious Nissl granules and are normal. Contrast this limited distribution of retrograde changes with the almost universal effects after evacuation of the eyeball (fig. 6). Cresyl fast violet.  $\times 176$ .



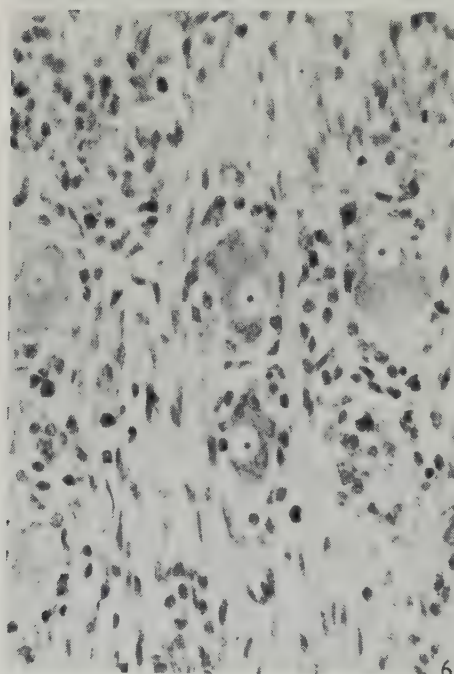




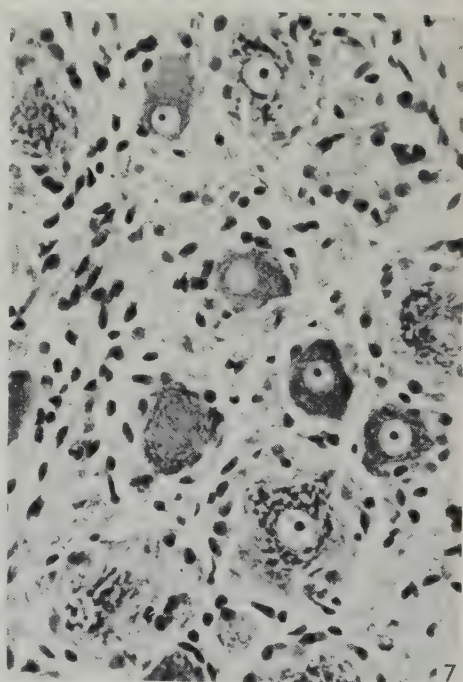
4



5



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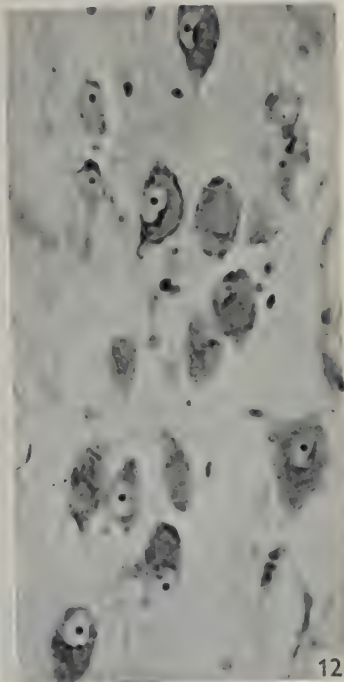
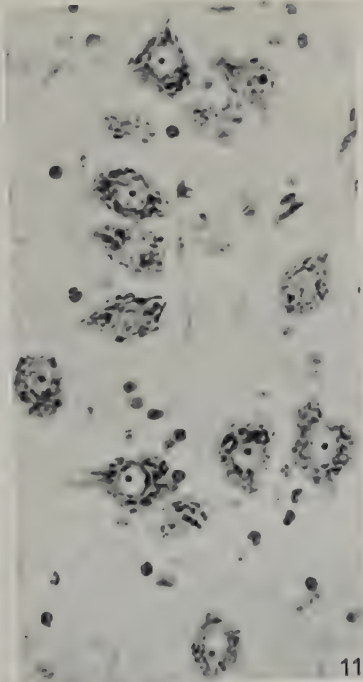
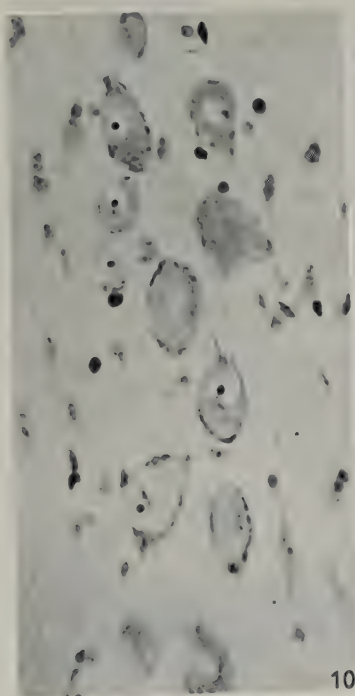
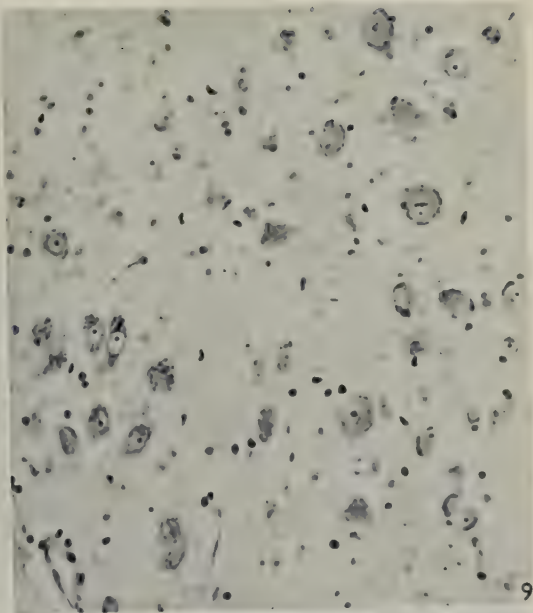






PLATE 3

- Fig. 8. This field show the right and left Edinger-Westphal columns in the midbrain of a monkey subjected to right ciliary ganglionectomy. Even at this magnification some of the cells in the right nucleus display a loss of Nissl granules. Cresyl fast violet.  $\times 80$ .
- Fig. 9. A field from the same animal as in the preceding figure, but at a more rostral level. Cells in the right and left halves of the antero-median nucleus can be compared. Those on the left contain Nissl granules, whereas almost all the cells on the right display marked retrograde degeneration (chromatolysis, swelling, and nuclear eccentricity). Cresyl fast violet.  $\times 80$ .
- Fig. 10. Chromatolysis in the nerve cells of the antero-median nucleus of another monkey, 11 days following ciliary ganglionectomy. All the cells in the ipsilateral half of the nucleus showed such changes. Cresyl fast violet.  $\times 176$ .
- Fig. 11. Normal nerve cells of the monkey's antero-median nucleus. Compare with the adjacent figures and note the contrast. Cresyl fast violet.  $\times 220$ .
- Fig. 12. Chromophil stage of retrograde degeneration in nerve cells of the antero-median nucleus of a third monkey which had been subjected to ciliary ganglionectomy. Compare these cells with the normal neurones of this nucleus, shown in fig. 11. Cresyl fast violet.  $\times 176$ .

## A STUDY OF THE CHOROIDAL CIRCULATION OF THE EYE IN MAN

By KENNETH C. WYBAR

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In a monograph on the circulation and nutrition of the eye, Leber (1903) presented a detailed account of the gross anatomy of the choroidal vessels, and little further has been added to his description since that time. More recently, however, Ashton (1952) has introduced a new technique whereby the choroidal vessels may be studied in casts prepared by the intravascular injection of Neoprene latex. This procedure represents a distinct advance on dye-injection methods, because the cohesive and elastic qualities of Neoprene permit dissection of individual vessels from the main vascular mass, a particular advantage in the study of such a tissue as the choroid wherein the vascular structure is dense and complicated; this technique has, therefore, been followed in the present investigation.

Some features of the normal choroidal circulation, as demonstrated in cast preparations, have already been described (Ashton, 1952), but this paper is particularly concerned with the equatorial anastomoses between the anterior and posterior arterial groups, with the distribution of the choroidal arteries in relation to the important question of a segmental blood supply, with the formation of the main venous channels and with the zonal peculiarities of the chorio-capillaris.

### MATERIAL AND METHODS

#### (1) *Intravascular injection of Neoprene latex*

The human eye and the contents of the orbit, including the cavernous portion of the internal carotid artery, were removed in one mass by an intracranial approach at post-mortem. The ophthalmic artery, identified in the fresh specimen as it leaves the internal carotid artery, was irrigated through a glass cannula with tapwater for 60 min.; any large superficial vessels being ligated to prevent leakage and so allow thorough irrigation of the vessels of the eye. The specimen was then placed in a refrigerator for 12 hr. to promote haemolysis of any remaining blood clots, and finally irrigated with water.

Neoprene latex 572, diluted if necessary with water, was injected into the ophthalmic artery from a Woulff's bottle under a pressure of 5-10 lb., using an electric pump. Since Neoprene coagulates very rapidly, it is essential to start the injection at a high pressure, and this was achieved by releasing the clamps on the rubber tube on the cannula side of the Woulff's bottle a few seconds after switching on the pump, and by reducing the intra-ocular pressure before injection by a central corneal puncture wound.

The injected eye was fixed in 10 % formol saline and after removal of the cornea, lens, vitreous and retina, the uvea was carefully removed from the globe by cutting the vascular and nervous connexions which linked it to the sclera. The uvea was then



bleached in 2% potassium permanganate for 30–60 min., followed by washing in oxalic acid.

The intact choroid was floated into a glass hemisphere, designed to resemble the scleral cup in size and shape, and on withdrawing the fluid the choroid lined its inner surface. The choroid was examined with a wide-field stereoscopic microscope either by looking directly into the sphere, in which case the chorio-capillaris was clearly visible, or by observing through the under-surface of the sphere so that an unobstructed view was obtained of the larger choroidal vessels (Pl. 1, fig. 1).

The choroid was examined in greater detail by mounting it flat on a slide after cutting it radially in several places, but most of the observations described in this paper are based on an examination of individual vessels which were dissected under water from the main mass of the choroid with fine forceps, using a wide-field stereoscopic microscope (magnification  $\times 9$  to  $\times 35$ ) and direct illumination from a high-power low-voltage filament lamp.

(2) *Occlusion of a single short posterior ciliary artery prior to intravascular injection*

After the final irrigation described above, a weak solution of methylene blue was injected into the ophthalmic artery. The orbital tissues immediately behind the globe were prised apart until a short posterior ciliary artery lying near the optic nerve was exposed. The vessel was then ligated and severed between the ligatures. Neoprene, or indian ink, was injected into the ophthalmic artery and the uvea examined as previously described.

(3) *Injection of a single short posterior ciliary artery*

Around the central end of one short posterior ciliary artery, identified as described above, a ligature was placed to retract the artery and keep it taut during the insertion of a fine glass cannula through a small cut in the vessel wall. Indian ink was injected into the vessel, and the uvea subsequently examined as before.

## RESULTS AND DISCUSSION

The following observations all relate to the examination of twenty-seven human eyes which, as far as could be assessed, were normal in all respects.

*The arteries of the choroid*

The choroid is supplied by two arterial systems; the more posterior part by six to eight short posterior ciliary arteries which divide into many branches as they pierce the sclera around the optic nerve, and the more anterior part by the recurrent arteries which arise within the ciliary body from the major arterial circle of the iris (Pl. 1, fig. 2), from the long posterior ciliary arteries (Pl. 1, fig. 3), and from the anterior ciliary arteries (Pl. 1, fig. 4). The two systems approach one another at the equator of the eye, where they anastomose either through an intervening capillary network (Pl. 2, figs. 5, 6) or not uncommonly by direct continuity (Pl. 1, figs. 2–4; Pl. 2, figs. 6–8). When the anastomosis is a direct one the two arterial components may be recognized by the direction of their branches; those of the posterior system pointing forwards and those of the anterior one pointing backwards. This

free communication of the anterior and posterior blood supplies at the equator is contrary to the conception that the equatorial part of the choroid is an area of poor vascularity; a view put forward by Leber (1903), who considered that any intervening anastomosis is insufficient to allow the posterior system to maintain the circulation in the anterior part of the eye in the absence of a recurrent system.

In addition to the branches of the short posterior ciliary arteries which anastomose with the recurrent choroidal arteries at the equator, there are many branches running forwards in narrow sectors of the choroid, which terminate in arteriolar-capillary networks at some point between the peripapillary region and the equator. The short posterior ciliary arteries are not connected with one another by arterial branches except in the most posterior part of the choroid, so that to a certain extent they have the appearance of end-arteries. If they are true anatomical end-arteries, however, it would follow that occlusion of a single short posterior ciliary artery, prior to the injection of the eye by way of the ophthalmic artery, should result in a filling defect in the appropriate sector of the choroid. Although this occurred in three eyes (two injected with Neoprene and one with indian ink), in four other eyes (two injected with Neoprene and two with indian ink) there was complete filling of the choroid. Furthermore, more than two-thirds of the choroid, ciliary body and major arterial circle of the iris became filled in two eyes which were injected with indian ink through a single short posterior ciliary artery.

From an anatomical point of view the short posterior ciliary arteries cannot, therefore, be regarded as true end-arteries, because each one is in communication with the anterior uveal circulation, including the major arterial circle of the iris, through the free anastomosis between the two circulations at the equator, and consequently in communication with the other sectors of the choroid through the recurrent choroidal arteries. Furthermore, the chorio-capillaris, which forms a continuous series of capillary tubes on the inner surface of the choroid, provides another channel of communication between adjacent arteries. These findings are contrary to any conception of a rigid segmentation of the choroidal arteries, despite the occurrence of a sectorial filling defect in three out of seven eyes in which a single short posterior ciliary artery was occluded prior to the injection of the ophthalmic artery. It seems likely that the occurrence of such a filling defect is largely an expression of the success of the intravascular injection technique. The injection material will pass initially into the arteriolar-capillary network in that part of the choroid supplied by the patent vessels, but, provided the injection is continued beyond this point, the zone dependent on the occluded vessel becomes filled by the alternative pathways.

#### *The veins of the choroid*

The four main vortex veins in the region of the equator of the eye are formed by the junction of venous tributaries which converge towards them from neighbouring parts of the choroid. Occasionally a subsidiary vortex vein is formed which leaves the globe independently of the main venous exits (Pl. 3, fig. 9). Some of the venous tributaries pass directly forwards from their origin in the chorio-capillaris to the nearest vortex vein, but others pass backwards towards the peripapillary region before sweeping round to join the main vessels (Pl. 3, fig. 9). This results in a well-marked concentration of venules in the posterior part of the choroid.

Kiss & Orbán (1951) demonstrated swellings (bulbiculi) in the walls of the choroidal venules which they believe to be the site of arterio-venous anastomoses through which the choroidal circulation is controlled. Ashton (1952), however, showed that the bulbiculi occur simply as a result of compression of the venule by the overlying arteriole, and that they are not the site of any arterio-venous communication. This view is confirmed in this further study in which no evidence has been found of any direct arterio-venous anastomoses in any part of the choroid.

### *The capillaries of the choroid*

The capillaries, which form a single layer on the inner surface of the choroid, are not uniform in calibre throughout the chorio-capillaris. Posteriorly, they are smaller with a closer intercapillary network and anteriorly they become wider with a more open network (Pl. 3, fig. 10). The capillaries are, therefore, more dense in that part of the choroid which lies under the macula as compared with capillaries in a more peripheral part of the choroid, but there is no detectable structural difference between the chorio-capillaris underlying the macula as compared with any other part of the chorio-capillaris situated at an equivalent distance from the optic disc. It would appear, therefore, that the density and calibre of the capillaries within the chorio-capillaris are purely an expression of the distance of the capillaries from the optic disc, and that there is no evidence to support the generally accepted belief of Leber (1903), and Nettleship (1903), that the chorio-capillaris which supplies the macular region has distinctive anatomical features.

### SUMMARY

1. The technique is described for the preparation of casts of the choroidal vessels of the eye by intravascular injection of Neoprene, the cohesive and elastic properties of which permitted the study of individual vessels dissected from the main vascular mass.
2. The meeting place of the posterior choroidal circulation (short posterior ciliary arteries) and anterior choroidal circulation (recurrent branches from the major arterial circle of the iris, long posterior ciliary artery and anterior ciliary artery) may be marked by an intervening capillary network, but many of the vessels in the anastomosing circulations are in direct continuity with one another.
3. The short posterior ciliary arteries are segmentally arranged, and each branch supplies a localized zone of the choroid with an arteriolar-capillary network, but there is no anatomical evidence for the conception that the short posterior ciliary arteries are true end-arteries.
4. No arterio-venous anastomoses have been found in any part of the choroid, although these have been postulated by other workers.
5. The size and density of the vessels in the chorio-capillaris vary in different regions of the choroid according to their distance from the optic disc, but there is no evidence for the view that the chorio-capillaris underlying the macula has distinctive anatomical features.

I should like to thank Dr Norman Ashton for introducing me to the technique of Neoprene injection and for his encouragement and advice throughout this investiga-



tion. I am indebted to Messrs G. Knight and A. McNeil and to the Medical Illustration Department at the Institute of Ophthalmology for their help in the preparation of the casts and the photographs.

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#### EXPLANATION OF PLATES

*Abbreviations:* A, cut end of anterior ciliary artery; E, equator; L, cut end of long posterior ciliary artery; M, major arterial circle of iris; O, region of optic disc; R, recurrent choroidal artery; S, short posterior ciliary artery; V, venule; VO, vortex vein; VS, subsidiary vortex vein.

##### PLATE 1

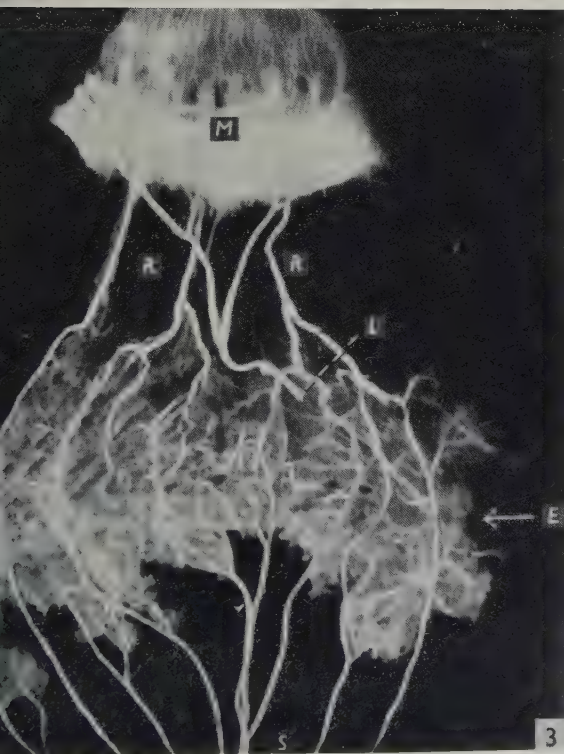
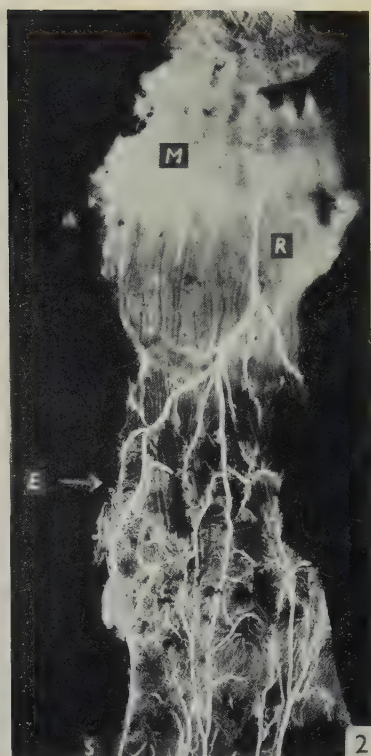
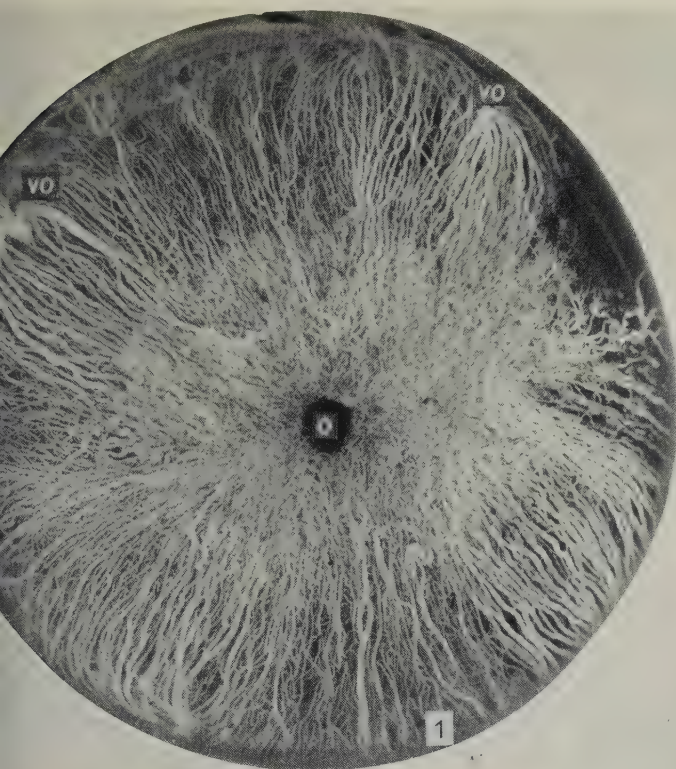
- Fig. 1. Neoprene cast of the intact choroid mounted in a glass sphere and viewed from without—larger arteries and veins nearest camera.  $\times 4$ .  
 Fig. 2. Neoprene cast of a strip of uvea showing, above, a recurrent choroidal artery arising from the major arterial circle of the iris, and, below, the terminal parts of several short posterior ciliary arterial branches. Note the direct continuity of the two arterial systems at the equator although in places there is an intervening capillary network. (Dissected specimen.)  $\times 8$ .  
 Fig. 3. Neoprene cast of a strip of uvea showing, above, three recurrent choroidal arteries arising from a long posterior ciliary artery, and, below, the terminal parts of several short posterior ciliary arterial branches. Note the direct continuity of the two arterial systems at the equator although in places there is an intervening capillary network. (Dissected specimen.)  $\times 10$ .  
 Fig. 4. Neoprene cast of a strip of uvea showing, above, a recurrent choroidal artery, arising from an anterior ciliary artery, and below, the terminal parts of several short posterior ciliary arterial branches. Note the direct continuity of the two arterial systems at the equator although in places there is an intervening capillary network. (Dissected specimen.)  $\times 8$ .

##### PLATE 2

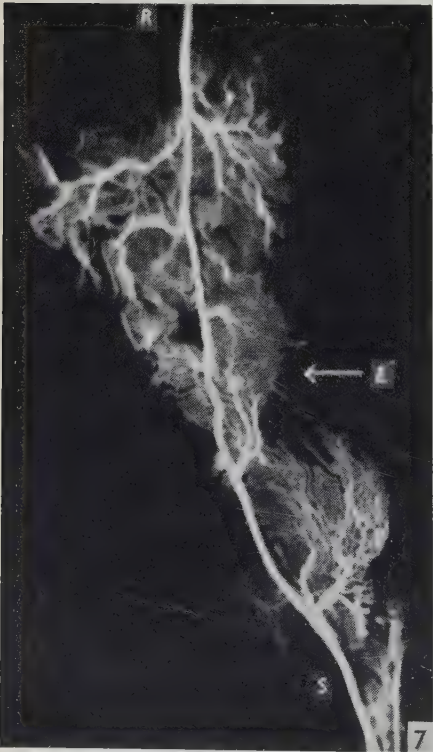
- Fig. 5. Neoprene cast of the terminal parts of a recurrent choroidal artery, above, and of a short posterior ciliary arterial branch, below, showing an intervening capillary network at the equator. (Dissected specimen.)  $\times 19$ .  
 Fig. 6. Neoprene cast of the terminal parts of three recurrent choroidal arteries, above, and of two short posterior ciliary arterial branches, below, showing at the equator an intervening capillary network between the two systems on the right, and direct continuity of the systems on the left. (Dissected specimen.)  $\times 16$ .  
 Fig. 7. Neoprene cast of the terminal parts of a recurrent choroidal artery, above, and of a short posterior ciliary arterial branch, below, showing direct continuity of the two arterial systems at the equator. Note the opposing directions of the branches of the two arteries. (Dissected specimen.)  $\times 14$ .  
 Fig. 8. Neoprene cast of the terminal parts of two recurrent choroidal arteries, above, and of a short posterior ciliary arterial branch, below, showing the direct continuity of the two arterial systems at the equator. (Dissected specimen.)  $\times 19$ .

##### PLATE 3

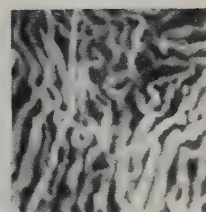
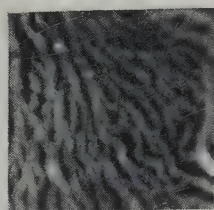
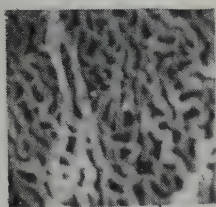
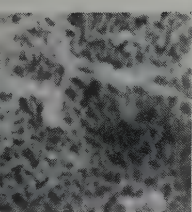
- Fig. 9. Neoprene cast of the choroidal veins showing two main and one subsidiary vortex vein system. Note the sweeping round of certain venules in the peripapillary region as they run to the vortex veins. (Dissected specimen.)  $\times 6$ .  
 Fig. 10. Neoprene cast of the chorio-capillaris showing the increase in calibre of the capillaries and decrease in density of the intercapillary meshwork in passing from the posterior to the anterior parts of the choroid, that is, from areas marked 1 to 4. (Dissected specimen.)  $\times 5$  and  $\times 24$ .













# THE FORMATION OF VILLI FOLLOWING ARTIFICIAL LESIONS OF THE MUCOSA IN THE SMALL INTESTINE OF THE CAT

BY R. M. H. McMINN AND J. E. MITCHELL

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## INTRODUCTION

The mechanism of growth and the reparative power of tissues were reviewed in 1894 by Bizzozero, who included epithelial coverings among those tissues which could continue to multiply throughout the life of the individual. The fact that the epithelial cells of the alimentary tract could be included in that category was well appreciated, and numerous investigators, both before and after that time, have demonstrated the regeneration of intestinal epithelium following a variety of lesions. In recent decades the stomach and duodenum have been selected for intensive study by many workers, in view of the problems of peptic ulceration in man. In the course of a series of studies on Brunner's glands, Florey & Harding (1935) investigated the healing of artificial defects of the duodenal mucosa of the cat. In these studies it was found that not only did complete epithelialization of the lesion occur but new villi were formed. In this way an almost normal pattern of mucosal architecture was restored with the exception of the muscularis mucosae which did not regenerate. Thus reconstitution of part of an organ occurred as well as regeneration of epithelium. These findings were contrary to views then current on the extent of healing of intestinal lesions, which was commonly believed to be confined to a simple epithelial repair overlying scar tissue. Healed lesions of the small intestine (excluding the duodenum) in man are not commonly examined, because their presence is not suspected or their site is not known. Brown & Sampson (1930), however, have cited evidence to the fact that in certain cases of intestinal tuberculosis, especially where the ulceration has been superficial, a complete epithelial covering has been formed with new, though irregular, gland formation.

The present work was carried out to investigate the mode and extent of villous formation at the site of artificial lesions of the mucosa in the ileum of the cat. At the same time the opportunity was taken to make observations in a number of the experimental animals on the mitotic activity of the regenerating epithelium, using colchicine.

## MATERIAL AND METHODS

All experiments were performed on healthy adult cats. An 'artificial ulcer' was created in the small intestine by operation, and the site of this lesion was examined histologically at various post-operative periods. In a number of animals, colchicine was used to arrest mitosis.

### *Operative technique*

The cats were starved for a pre-operative period of 24 hr. They were anaesthetized with pento-barbitone given intraperitoneally, following which the abdominal cavity was opened and a loop of small intestine was brought out through the wound. At



a site 45 cm. proximal to the ileo-caecal junction, an incision 2.5 cm. in length was made along the anti-mesenteric border, and the mucosal surface of the mesenteric border was protruded through the incision by a finger of the assistant. A square, approximately 1 sq.cm. in size, was mapped out on the protruding mucosa surface by four incisions. From this delimited area, the whole thickness of mucosa and submucosa was removed. During this part of the procedure it was found that at the level of the inner circular muscle of the intestinal wall there was a plane of easy cleavage which facilitated the stripping off of the overlying mucosa and submucosa. The intestinal and abdominal wounds were then closed, a continuous Connell suture being used for the intestinal wall. The animals were starved for a post-operative period of 24 hr. before the gradual resumption of normal feeding.

The lesion thus created in the small intestine is referred to throughout this paper as an 'artificial ulcer'.

For the study of mitotic activity in regenerating epithelium, colchicine was used to arrest mitosis in metaphase. The dose was 0.25 mg./kg. of body weight, given intraperitoneally in aqueous solution 5 hr. before death. Using this dosage and time interval it was found that dividing nuclei were in the stage of metaphase and later phases of the mitotic cycle were not seen (Pl. 2, fig. 16).

The animals were killed 1, 2, 3, 4, 7, 10, 14, 42 and 100 days after operation, four animals being used for each time period.

#### *Histological technique*

The portion of intestine bearing the ulcer was removed, opened along the original incision and pinned out on cork prior to fixation in 80 % alcohol. After embedding in paraffin wax, sections  $7\mu$  thick were cut and stained with either haematoxylin and eosin or with iron haematoxylin and picrofuchsin. Some sections were stained with acid fuchsin and light green. For the demonstration of mucin the Gomori (1946) technique was used following preliminary digestion with ptyalin (saliva).

Mitotic counts were carried out by counting the resting and dividing nuclei of epithelial cells. The number of nuclei in arrested mitosis was then expressed as a percentage of the total number, at least 2000 nuclei being included in any single count. The counts were carried out using a square mask in one of the oculars of a binocular microscope, the nuclei being counted in contiguous fields from the bases of crypts to the tips of villi. Although mitosis normally occurs only in the epithelium which lines the crypts, the percentages expressed refer to the whole epithelial covering including that of the villi. Counts were undertaken in the epithelium of undisturbed mucosa in regions immediately adjacent to the original ulcer margin, where both crypts and villi were cut approximately throughout their length. Thus it was possible to gauge the mitotic activity of the epithelium at comparable sites during different stages of the healing process. Control counts were carried out on sections cut at  $7\mu$  and stained with haematoxylin and eosin, taken from sites 15 cm. distal to the ulcers. Random fields were chosen and counts undertaken only where the crypts and villi were sectioned approximately throughout their length.

## RESULTS

### *Gross appearance*

The site of operation in the intestine was identified externally by unabsorbed catgut, and a varying number of peritoneal adhesions were usually present. In the early post-operative period, the floor of the ulcer resembled reddish granulation tissue, and by the end of the second week a shallow depression with the colouring of the surrounding mucosa was the only evidence of the lesion. There was nothing in the mucosal surface of the 42- and 100-day specimens to suggest that there had been operative interference, and portions of unabsorbed catgut alone indicated the region to be examined histologically, although on palpation a slight thickening of the intestinal wall could be appreciated.

### *Histological examination*

Histological examination of the artificial ulcer 24 hr. after operation (Pl. 1, fig. 1) revealed the presence of blood clot, with round cell infiltration, covering the floor of the ulcer. There was some oedema of the underlying muscle. The periphery of the clot was covered with a single layer of flattened cells with centrally placed nuclei (Pl. 2, fig. 8). When traced towards the margin of the ulcer these cells gradually became taller with more basally placed nuclei and were in continuity with the normal columnar epithelium. After 2 days the cells had extended for a distance of approximately  $500\mu$  towards the centre of the ulcer, and the mucosal margin had fallen towards the floor of the ulcer (Pl. 1, fig. 2). An increasingly wide area of ulcer was covered during subsequent days by this single layer of cells which often burrowed beneath the clot. By the fourth day the flattened type of cell was no longer seen; all were cuboidal or low columnar (Pl. 2, fig. 9), and at the periphery of the ulcer occasional goblet cells were seen. Shallow depressions now appeared in this hitherto level covering at the periphery, and examination after 7 and 10 days suggested that these were deepening to form pits, the deepest being those nearest the ulcer margin (Pl. 2, fig. 13). Goblet cells were now more frequent.

At the time when the cellular layer first became pitted (4 days) a number of rounded cyst-like spaces were seen at a deeper level (Pl. 2, fig. 15). These were lined with a single layer of cells similar to those on the surface—usually columnar but in places much flattened. The examination of serial sections revealed that these spaces were continuous with the lumen of the intestine at the bases of the pits, of which they appeared to be the dilated terminations.

While the above changes were taking place, granulation tissue had been accumulating beneath the clot (Pl. 1, fig. 3) and organization was occurring with the formation of a varying amount of fibrous tissue in the base of the ulcer. The organizing tissue showed signs of considerable vascularity, with the larger vessels coursing towards the ulcer surface. The muscularis mucosae could be seen to end abruptly where it had been cut at the original margin of the ulcer.

By the end of the second week almost the whole ulcer had become covered by a single layer of cells (Pl. 1, fig. 4); the surface in the central area remained flat, the cells being of columnar type (Pl. 2, fig. 14), while towards the periphery the

deepening depressions and pits gave rise to a pattern of increasingly long finger-like projections clothed with tall columnar cells and many goblets (Pl. 2, fig. 12).

After 42 days the central area of the ulcer was no longer flat but showed irregularities similar to the projections already well-defined peripherally (Pl. 1, figs. 5, 6).

Sections of ulcers examined 100 days after operation revealed a picture very similar to that of normal small intestine (Pl. 1, fig. 7). Narrow finger-like projections with intervening pits, covered by closely packed columnar cells, many of goblet type, were seen overlying a 'submucosa' composed of collagenous tissue of varying density. The muscularis mucosae was still seen to end abruptly at the original ulcer margin (Pl. 2, fig. 11), and although a few muscle fibres from the inner circular muscular layer of the intestinal wall intermingled with fibrous tissue in the submucosa in the original ulcer area, no muscle fibres were seen to course into the projections. Blood vessels, however, were prominent in the cores of the projections.

The results of mitotic counts are summarized in Tables 1 and 2. Counts at control sites indicated that 3.77 % of the total number of epithelial nuclei were in arrested mitosis 5 hr. after colchicine injection (Table 1). The figures obtained from the epithelium at the margins of ulcers at varying stages during the healing process (Table 2) suggest that mitotic activity was greater 24 hr. after operation than at other times, but the number of animals used for these observations was small and this apparent increase was not statistically significant ( $P > 0.05$ ).

#### DISCUSSION

The results show that, following repair, the site of the artificial ulcer eventually bears a close resemblance to normal intestine, the mucous membrane as a whole (with the exception of the muscularis mucosae) being reconstituted.

The regeneration of the epithelium itself proceeds in a manner which is well known. In epithelial coverings such as the epidermis and the mucous membrane of the intestine there is considerable regenerative activity even under normal conditions (Maximow & Bloom, 1952), in order to replace the frictional loss of cells to which these coverings are constantly subjected. In the case of the intestine, mitoses in the crypts are the source of the cells which make good this loss (Le Gros Clark, 1952). Leblond & Stevens (1948) showed that in the small intestine of the rat this physiological replacement occurred by a migration of cells from the bases of the crypts towards the tips of the villi, from which the cells were eventually shed. The process was continuous and was not significantly influenced by feeding. A similar process has more recently been demonstrated to occur among certain cells of the rat's stomach (Stevens & Leblond, 1953) but at a much slower rate.

The present studies indicate that crypts in the normal mucosa immediately adjacent to the ulcer margins are the source of the cells which become pushed over the floor of the ulcer. There is no evidence to suggest that any cells of the underlying granulation tissue are transformed in order to contribute to this epithelial layer, in a manner that has been described for endometrium (Papanicolaou, 1933). It has been said of epithelia in general that a solution of their continuity leads to 'a local dispersal of cells' which by the 'lessening of their mutual pressure appears to induce their multiplication' (Wright, 1950). To ascertain whether this could be applied to



Table 1. *Percentage of dividing nuclei in intestinal epithelium at control sites, 5 hr. after injection of colchicine*

	Total no. of nuclei	No. of nuclei in arrested mitosis	Percentage of nuclei in arrested mitosis	Average percentage
Cat 36	2220	74	3.33	3.74
	2612	108	4.13	
	2425	81	3.45	
	2510	98	4.06	
Cat 31	2293	85	3.85	3.58
	2223	84	3.95	
	2254	75	3.33	
	2190	70	3.19	
Cat 39	2298	92	4.00	3.99
	2375	95	4.00	
	2377	102	4.29	
	2101	77	3.67	

Mean of average percentage in arrested mitosis: 3.77.

Table 2. *Percentage of dividing nuclei in intestinal epithelium at ulcer sites 5 hr. after injection of colchicine*

	Days after operation	Total no. of nuclei	No. of nuclei in arrested mitosis	Percentage of nuclei in arrested mitosis	Average percentage
At ulcer margin					
Cat 63	1	2385	103	4.32	4.28
		2313	99	4.28	
		2439	100	4.10	
		2303	102	4.42	
Cat 31	1	2175	115	5.34	5.67
		2176	146	6.71	
		2181	105	4.82	
		2032	118	5.81	
Cat 64	1	2096	110	4.77	4.79
		2132	114	5.35	
		2101	103	4.90	
		2302	96	4.17	
Cat 50	2	2036	64	3.13	4.34
		2086	70	3.35	
		2165	146	6.75	
		2325	96	4.13	
Cat 24	4	2330	86	3.69	4.08
		2382	112	4.70	
		2697	98	3.63	
		2042	88	4.31	
Cat 49	7	2403	75	3.11	3.13
		2119	70	3.30	
		2366	71	2.91	
		2025	65	3.20	
Cat 39	100	2176	84	3.81	3.79
		2267	81	3.57	
		2154	91	4.22	
		2135	76	3.56	
At centre of healed ulcer					
Cat 36	100	2086	66	3.11	3.49
		2318	85	3.65	
		2132	75	3.52	
		2201	81	3.68	

intestinal epithelium, observations on the regenerative capacity of the epithelium were made by mitotic counts. These were carried out on animals receiving colchicine in preference to those to which it was not administered, as it was thought more desirable to compare mitotic activity in different sites over a period of time—in this case 5 hr.—than at the time of death. Although at first sight the figures (Tables 1 and 2) suggest that mitotic activity is greater 24 hr. after the production of the gap in the epithelial covering than at other times, this is not confirmed by statistical analysis. It would appear, therefore, that the presence of a defect in the intestinal epithelium does not serve as an added stimulus to the cell multiplication which is already in progress.

The observations with colchicine confirm that in the cat, as in other animals, epithelial mitosis is confined to cells in the crypts; only rarely is a dividing nucleus seen near the base of a villus, and none is found over the more distal areas of a villus or in the advancing epithelial layer. It is to be noted that multiplication does not occur among those cells which are in the fore-front of the advance to re-establish continuity. During the first 2 days the cells over the periphery of the ulcer bed have a flattened appearance, but at later stages this type of cell is no longer seen and they gradually assume a columnar form (Pl. 2, figs. 8–10). It is of interest to draw a parallel between the mechanism of repair taking place in the epithelium of the intestine and in that of the cornea. In these two tissues epithelial regeneration is comparable only in so far as the cells which cover the defect in the first instance do not show mitotic activity; the intestinal lesion is covered by newly formed cells from adjacent crypts, but in the case of stratified corneal epithelium the wound is made good by the migration into the gap of 'old' cells from the more superficial layers (Mann, 1932; Arey & Covode, 1943). With the formation of new crypts (see below) mitotic activity is brought nearer the centre of the ulcer, and arrested mitosis in their epithelial lining was first observed on the seventh post-operative day. It follows that up to this time the epithelial covering of the ulcer has been derived from 'old' crypts, and that subsequently the newly forming and newly formed crypts are responsible for the addition of cells to this covering, with the 'old' type then reverting to the physiological replacement of cells covering villi.

The precursors of new crypts are seen as early as the fourth post-operative day, as gradually deepening depressions of the epithelial covering into the underlying granulation tissue. The presence of cyst-like spaces under the epithelium near the ulcer margin at this time (Pl. 2, fig. 15) was an unexpected finding. It was first thought that these might be similar to the cysts seen by Giani (1907) in regenerating bladder epithelium, but the study of serial sections revealed their continuity with the lumen of the gut. In later stages they assume the slit-like character of the normal crypt. The flattening of their epithelial lining that is sometimes observed may be attributed to transient blocking of the narrow neck of the space, which may have caused a temporary increase in pressure.

It is by the deepening of primitive crypts that a villous configuration is first achieved, rather than by the upward growth of projections into the lumen of the gut (Pl. 2, fig. 13). While the epithelial downgrowths are developing, the underlying granulation tissue is becoming organized. Such organizing tissue as remains between the deepening crypts is destined to become the stroma of villi. In contrast to epi-

thelial cells, which are derived from parent cells as a result of considerable mitotic activity, only very rarely is a cell in division seen in the differentiating stroma; here movement of cells rather than mitosis is the keynote. By the end of 2 weeks villi approaching the normal pattern are readily identified (Pl. 2, fig. 12). Subsequent elongation and the development of a characteristic vascular pattern in the core give rise to the typical finger-like appearance of the fully formed villus. The site of the lesion after 100 days demonstrates the fact that a restoration of mucosal architecture closely resembling the normal has occurred. Crypts and villi have been reconstituted, and although the crypts are less deep and the villi less closely packed than is usual, individual villi appear similar to those in the surrounding mucous membrane, except that they are not seen to contain prolongations of musculature from the muscularis mucosae. The cut edge of the muscularis mucosae can be identified at the original margin of the ulcer at all stages of the healing process examined (Pl. 2, fig. 11). The absence of regeneration in this component of the intestinal wall has been repeatedly observed both in animals and in man.

Although by the second day the mucosal margin has fallen towards the floor of the ulcer (Pl. 1, fig. 2) there is no other evidence to indicate that the defect is being made good by a sliding of the mucosa, with or without the submucosa, towards the ulcer centre. The new submucosa contains a greater proportion of collagenous fibres than in regions not subjected to operative interference. As in the case of the cat's duodenum (Florey & Harding, 1935) the lesion has healed not only by epithelialization but by a reconstitution of mucous membrane comprising both crypts and villi. In both duodenum and ileum the new villi are less closely packed than in normal mucosa; in the duodenum Brunner's glands also regenerated but the muscularis mucosae, as in the ileum, showed no evidence of repair.

These experiments indicate that it is possible for a very complete repair of the intestinal mucous membrane to take place. In these experimental wounds there is a minimum of inflammatory reaction unaccompanied by the infective elements which so often complicate the healing of disease processes. The endarteritis of pathological lesions of the alimentary tract mitigates against an optimum response, and it seems probable that the relatively high vascularity of the experimental lesion contributes in no small measure to the degree of repair that can ultimately be achieved.

#### SUMMARY

1. The healing of mucosal lesions in the small intestine of the cat has been studied following the operative removal of areas of mucosa and the underlying submucosa, in order to examine the mode and extent of villous formation at the site of such a lesion.

2. Epithelialization occurs by cell division in the crypts; in the early stages in the crypts of the normal mucosa at the ulcer margins and later in the new crypts formed in the floor of the ulcer. Mitosis does not occur in the advancing epithelial layer. Histological observations using colchicine suggested that mitotic activity in the epithelium was greater 24 hr. after the production of a gap in the epithelial covering than at other times, but this apparent increase was not found to be statistically significant.



3. The precursors of new crypts and villi are first seen on the fourth post-operative day. Downgrowths of the epithelial surface into the underlying granulation tissue gradually deepen to form the crypts; the organizing tissue that remains between the developing crypts becomes the stroma of new villi.

4. Mitosis is first seen in newly forming crypts on the seventh post-operative day.

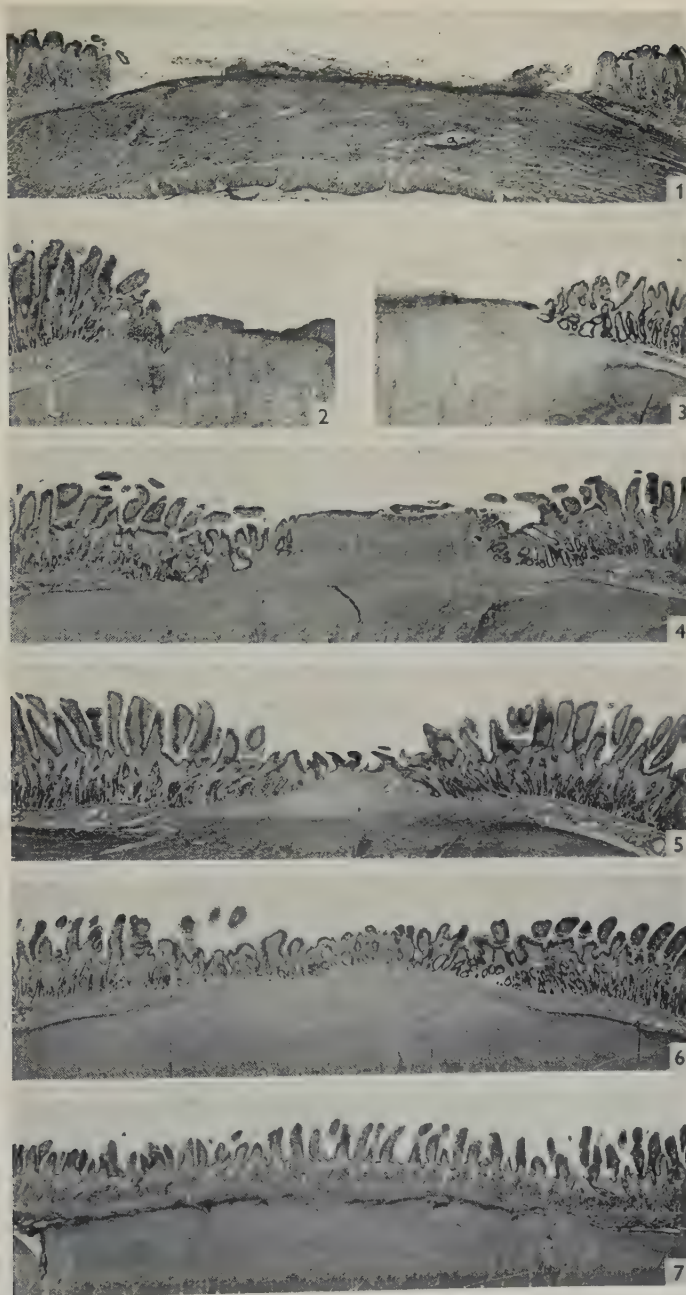
5. The muscularis mucosae does not regenerate and the submucosa contains a greater proportion of collagenous fibres than normal.

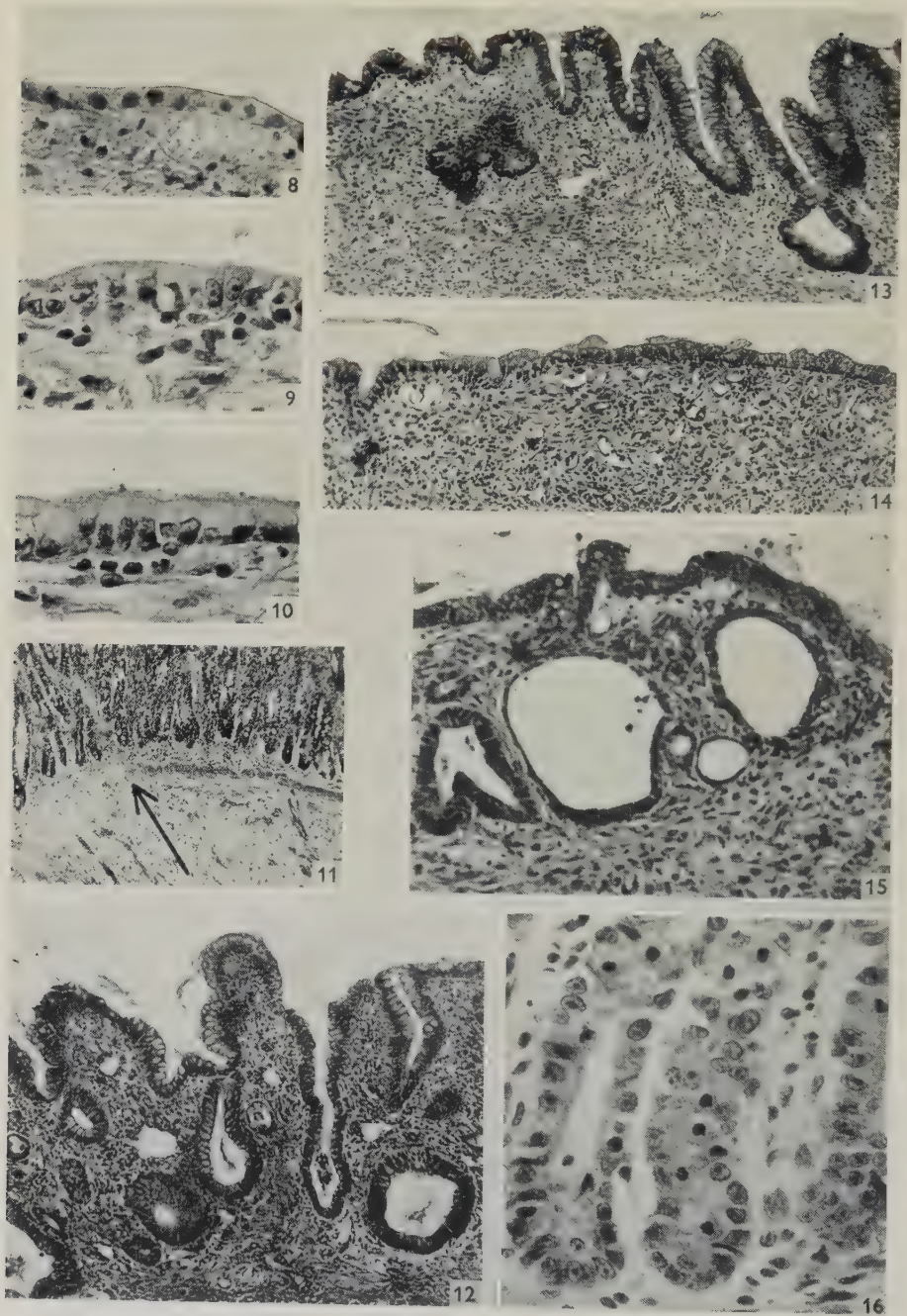
6. After 100 days the reconstituted villi are less closely packed and the crypts less deep than is usual, but the pattern of mucosal architecture bears a close resemblance to the normal.

We wish to express our thanks to Prof. Francis Davies, Dr F. R. Johnson and Dr F. J. Ebling for their interest and helpful advice during this work. We are indebted to Mr J. H. Kugler and Mr J. Morrill for technical assistance and for the preparation of the photomicrographs. Part of the expenses incurred in carrying out this work has been borne by a grant from the Medical Research Fund of the University of Sheffield, and for this we also desire to express our thanks.

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McMINN AND MITCHELL—FORMATION OF VILLI FOLLOWING ARTIFICIAL LESIONS OF THE MUCOSA



EXPLANATION OF PLATES

PLATE 1

All sections were stained with haematoxylin and eosin. Magnification,  $\times 11$ .

- Fig. 1. Section through the centre of an artificial ulcer 24 hr. after operation, showing that mucosa and submucosa have been removed, leaving the inner circular layer of muscle and overlying blood clot to form the floor of the ulcer. A thin layer of epithelium is seen over the periphery of the floor on the right.
- Fig. 2. Ulcer margin after 2 days. The edge of the mucosa has fallen towards the floor of the ulcer.
- Fig. 3. Ulcer margin after 7 days. Granulation tissue has been accumulating in the floor of the ulcer, and new crypts and villi are forming at the periphery.
- Fig. 4. Section through the centre of an ulcer after 14 days. The epithelial covering is almost complete and remains flat in the central area. Towards the periphery new crypts and villi are seen.
- Fig. 5. Section through the centre of an ulcer after 42 days. The whole surface shows irregular projections into the lumen of the gut, with some underlying fibrous tissue.
- Fig. 6. After 42 days, from the same ulcer as fig. 5 but nearer the periphery. The formation of crypts and villi is more clearly defined.
- Fig. 7. Section through the centre of an ulcer after 100 days. Crypts and villi have been re-constituted. The crypts are less deep than normal and the villi less closely packed. The submucosa is more fibrous than normal.

PLATE 2

All sections were stained with haematoxylin and eosin, except fig. 11 where the stain was acid fuchsin and light green.

- Fig. 8. 1 day. Flattened cells with centrally placed nuclei are overlying the periphery of the ulcer.  $\times 380$ .
- Fig. 9. 4 days. The cells covering the periphery of the ulcer are now cuboidal or columnar.  $\times 380$ .
- Fig. 10. 7 days. In the advancing epithelial layer, the cells are becoming more columnar (towards the left) as the ulcer margin is approached. Compare with figs. 8 and 9.  $\times 380$ .
- Fig. 11. 100 days. The section, from the region of the original ulcer margin, shows the abrupt termination of the muscularis mucosae (indicated by the arrow).  $\times 40$ .
- Fig. 12. 14 days. Near the ulcer margin deepening crypts are seen with the accompanying formation of villi. Compare with fig. 13 which shows earlier stages of this process.  $\times 75$ .
- Fig. 13. 7 days. The depressions in the epithelial covering have deepened to form pits as the ulcer margin is approached (towards the right).  $\times 70$ .
- Fig. 14. 14 days. The even columnar epithelial covering near the centre of the ulcer is shown, with early crypt formation beginning on the left, nearer the ulcer margin. From the same section as fig. 4.  $\times 95$ .
- Fig. 15. 4 days. Cyst-like spaces are seen near the ulcer margin under the epithelial covering.  $\times 150$ .
- Fig. 16. Normal mucosa showing typical colchicine mitoses in crypts.  $\times 380$ .

## REVIEWS

*Primates. Comparative Anatomy and Taxonomy. I. Strepsirhini.* By W. C. OSMAN HILL. (Pp. xxiii+798; 34 plates and 199 text-figures; £5. 5s.). Edinburgh University Press.

All those concerned with studies of the Primates will agree that there has long been the need for a comprehensive and systematic reference work on this mammalian Order, for during the last few decades there has been a great accumulation of knowledge about Recent and fossil types which is only to be found in scattered literature. Dr Osman Hill is now engaged in the strenuous task of compiling such a reference work, and the first-fruits of his labours have appeared in the form of a volume on the lemurs. Clearly this is the product of many years of intensive study, and it is also quite obviously based on personal researches of unusual variety. In it will be found such a complete, systematic, and detailed account of the comparative anatomy of the lemurs that there is no previous monographic work on the Primates with which it can properly be compared. It is richly illustrated with text-figures, maps and plates and includes descriptions not only of the existing lemurs but also of all the fossil types so far known. We have not attempted, for this review, to read through the whole volume page by page; indeed that would be a task of very considerable magnitude.

It would no doubt be quite impossible in such a comprehensive monograph as this to avoid minor errors here and there, but we must confess that a sampled perusal suggests that these are exceptionally few. To be sure, we would be prepared to argue with the author about certain statements, e.g. that no Eocene Primates have yet been found in Asia, that the Oligocene Primates belong to existing African groups, that Wallace's line lies west of the Philippines, and that all living Primates are confined to tropical regions; and the statement that the macaque is the only Primate in Europe reads oddly when one thinks of the population of *Homo sapiens*. But these are seemingly more of the nature of technical slips than errors. We would venture a protest, also, against the reference to (and illustration of) Neumayer's 'endocranial cast' of *Adapis parisiensis*, in which a cast of the nasal cavity was mistaken for a quite ridiculously large olfactory bulb. The perfect endocranial cast, prepared some years ago at the Natural History Museum, South Kensington, and figured in this *Journal* (Vol. 79, 1945), makes it clear that even in this Eocene lemur the olfactory bulb had already begun to undergo the reduction characteristic of Primates generally. Probably, however, the main criticism with which Dr Hill's admirable book will have to contend is his system of classification. For a taxonomic definition of the Primates, for example, he relies on Mivart's diagnosis of eighty years ago. But while this may be adequate when consideration is limited to the static end-products of Primate evolution represented by most of the existing species of to-day, it can hardly be applied in palaeontological studies; indeed, apart from the character 'three kinds of teeth, at least at one time of life', none of Mivart's criteria can be applied to the majority of fossil lemurs which Dr Hill himself includes in his systematic account. We would suggest, also, that he seems to be not quite consistent in excluding the Tupaioidea from the Primates on the grounds that 'the time-honoured diagnostic characterization of the Primate order has to be stretched almost beyond recognition in order to accommodate them', for, in fact, it has to be stretched very much further still to accommodate forms such as the plesiadapids which Dr Hill does include. (Incidentally, *Megachiromyoides* is now regarded by Stehlin and Schaub as not a plesiadapid at all, but a rodent synonymous with the genus *Ailuravus* described many years ago.) Dr Hill adopts Pocock's taxonomic grouping of the Primates, with the lemuriforms and lorisiforms in the grade Strepsirhini and the tarsiiforms and the higher Primates together in the grade Haplorhini. This is, of course, a possible alternative to the scheme of classification which is now more generally adopted (and here we would join issue with the statement that Pocock's classification 'has since been almost universally

accepted'), but it is bound to lead to some confusion. No doubt, the disadvantage of Pocock's scheme is that it seems to place too much reliance on a single external and superficial character—the construction of the rhinarium, and unfortunately we do not know (and are never likely to know) what the rhinarium was like in fossil prosimians. It is also a fact that, with increasing knowledge of the skeletal anatomy of fossil prosimians, it is becoming more and more difficult to differentiate between the early lemurs and tarsiers. For example, Hürzeler's study of *Necrolemur* has quite recently demonstrated that the tympanic ring is actually enclosed within the bulla—a character which hitherto has been regarded as diagnostic among eutherian mammals of the lemuriforms (and the tree-shrews). It is for very good reasons of this sort, of course, that Dr G. G. Simpson, in his attempt at a natural classification, groups the tarsiers and lemurs in the common sub-order, Prosimii. This question of the taxonomic subdivision of the Primates is a vexed one, and will probably remain so until a more complete palaeontological record can demonstrate phylogenetic relationships with greater certainty. But, for the moment, it would undoubtedly make for clarification if primatologists could agree, on a provisional basis and without the need for *final* acceptance, to use a classification already in common use.

This is not intended to be a 'critical' review in the ordinary sense. Indeed, in the reviewer's opinion, Dr Hill's book does not call for such a critical review—it is much too good for that. There can be no doubt at all that it will become the standard work of reference on the anatomy of the lemurs, and, if the succeeding volumes maintain the very high standard of the present one, the author will have every reason to be proud of his achievement.

W. E. LE G. C.

*An Introduction to Functional Histology.* By G. H. BOURNE. (Pp. 208; 98 illustrations; 9 × 6½ in. 21s.) London: J. and A. Churchill Ltd. 1953.

Dr Bourne is to be congratulated on producing a book whose main purpose is to help students to realize that the tissues which they see under the microscope contain cells which have, in their natural surroundings, an active and often varied existence, in which the interplay between the constituents of the cell and those of its surroundings may cause considerable structural and functional modifications. So far, so good. This is a truly admirable purpose, and in many parts of the book the author achieves his aim in a most stimulating manner. As the author points out, however, the book is necessarily uneven because of the uneven incidence of available information, and it is possible for the reader to suggest that there are several places in the book where the subject-matter may owe its limitations to causes other than the lack of information available in libraries.

Excellent as is the aim of the book in one direction, in another it is definitely bad. The book is presumably intended for serious-minded or advanced students who wish to supplement the ordinary text-books, particularly on the functional side. From this point of view one or two of the sections, e.g. that on the special senses, could well be omitted as they do nothing in this direction; but, far more serious than that, the student is not encouraged to enquire further into any subject for himself. No references are given to the sources of information and the only way of finding out more about any subject is, if it happens to be illustrated by a figure, to refer to a rather awkwardly arranged list of authors and publications to whom the author is indebted for the permission to reproduce the figures, and then to guess in what book or journal further information might be found. Furthermore, the book abounds in such phrases as 'It is said that...', 'For many reasons it is difficult to accept that...', 'Many authors believe...' and so on. Surely, it would have been better to cut out those sections of the book which contribute little new, and to use the space thus released to give the evidence for and against any controversial statement, so that the student may attempt to formulate an opinion on the matter for himself. After all, the main purpose of education is to persuade the student to think for himself and to evaluate evidence, rather than to convert him into a walking encyclopedia filled with *ex cathedra* statements destined to become obsolete within the year.

The illustrations are mostly very good and stimulating, though their significance is



sometimes rather inadequately stressed in the text. There are several minor errors. For example, on p. 153 reference is made to 'cones' when 'rods' are clearly intended, and I trust that the author will forgive me if, after reading p. 62, I am tempted to comment as follows:

'Twas Ebert helped Sir Howard Florey  
(E berth's on a different storey)  
So, if you would de-bunk our story,  
You really shouldn't call me Flory.'  
Said Floorey to Born.  
*Sed floreat Bourne.*

E. N. WILLMER

*Introduction to Dental Anatomy.* By JAMES H. SCOTT and NORMAN BARRINGTON BRAY SYMONS. (Pp. viii + 292; figures 172;  $8\frac{1}{2} \times 5\frac{1}{2}$  in. 35s.) Edinburgh and London: E. and S. Livingstone Ltd. 1952.

This book has obviously been written with the intention of providing for the student in a reasonable compass, matter which is treated discursively in other text-books on the subject. On the whole, the authors have succeeded in this aim but inevitably the necessary condensation has led to some imbalance in the presentation. This is exemplified by the relatively slight importance given to root growth in the process of eruption. Moreover, although the authors state in the preface that they have been somewhat selective and dogmatic in those matters in which there is uncertainty, it is surely carrying this too far to attribute the migration of the teeth during the period of facial growth largely to the effect of the mucous membrane attached to the teeth (p. 132), or to state without qualification that the surface epithelium of the oral mucosa is of ectodermal origin (p. 89) when the tongue is included in the subsequent description.

The section on dental histology is clear and well illustrated. The addition of pointer-lines to some of the figures would be an advantage in subsequent editions.

The section on general facial development is rather too condensed to be clear to students, but this is perhaps more the province of an ordinary text-book of anatomy. One would, however, have expected a very much greater emphasis in a book of this type on the functional aspects covered in chapter xvi. Here the actual process of chewing is relegated to two short paragraphs. The account of the growth of the face and jaws, although fairly short, is good. The same is true of the section on alveolar bone, but the original source on which diagrams, such as fig. 88, are based should be acknowledged. The part dealing with comparative anatomy is reduced in this book to a reasonable size, and the only criticism which might be made is the relatively small space devoted to the nearest neighbours of man.

The final general impression of the book is that its aim is excellent, that it is well produced and illustrated, although descriptions are, in places, rather involved and hard to follow. The authors would be wise in subsequent editions to reconsider the balance of the text and to expand some of the sections so as to avoid undue condensation presenting so dogmatic a picture that it becomes misleading to students. This would improve greatly a book which has, in condensing and selecting the subject-matter, filled a long-felt want for dental students.

J. WHILLIS

*Frazer's Manual of Embryology*, 3rd edition. Edited by J. S. BAXTER. (Pp. x + 488; 288 illustrations; 42s.) London: Baillière, Tindall and Cox. 1953.

*Frazer's Embryology* was first published in 1931, and, as the author then stated, was an attempt to give a descriptive account of human development arranged on a regional basis rather than in systems, as is usual in most text-books. A more important feature was that so much of the description was based directly on Frazer's own personal observations. The greater part of the book was, in fact, a description of his findings in a large series of embryos which he had collected over a long period of years. In this way the book acquired considerable importance as a record of personal research work, but, for the same reason, it was inevitable that, as a general text-book, it suffered from serious defects.

No one man, in his own personal work, can cover more than a small part of the extensive field of modern embryology. For the most part, this book consists of a careful, accurate and detailed account of the changes in form which occur in the different regions and organs of the human embryo during intra-uterine life, changes which can be studied in serial sections and illustrated by wax-plate reconstructions. Few workers can have studied material of this kind more thoroughly or more extensively, and the record is of permanent value. Such material, however, has its inherent limitations, and it is doubtful whether its detailed description can give the balanced view of present embryological knowledge which the elementary student requires; more advanced students will find the almost complete absence of references to the work of others a serious disadvantage.

In this 3rd edition, prepared by Prof. Baxter, some of the inadequacies of previous editions have been made good. Part I, on early and general development, has been re-written almost completely, and is a concise and clear account of modern knowledge in this field, with numerous references to original papers. The main part of the book has wisely been left substantially as it was written by Frazer. As the editor says, it is 'largely a record of the personal observations of one man extending over many years'. Extensive revision of such a record by anyone but the original author would be impossible; it must stand on its own merits, and these are considerable.

F. GOLDBY

*Histology.* By ARTHUR WORTH HAM. 2nd edition. (Pp. xix + 866; 7 colour plates, 518 illustrations; 10 × 7 in. 80s.) Philadelphia: J. B. Lippincott Company. 1953.

More information of direct interest to medical students is to be found in this text-book than in any other similar work in current circulation. One wonders whether English-speaking students in various countries will prefer its style and will gain more from it, because the author's approach is much more didactic than is usual for text-books on preclinical subjects. There is little doubt that the book will stimulate the young student to feel that Histology is an integral part of the study of structure and function and not an isolated and somewhat minor discipline.

Many problems arise in the writing of a comprehensive text-book: economy of size, sequence of chapters, the value of classification, the lack of an agreed terminology and the temptation to include up-to-date research, some of which may not stand the test of time. To begin with, this book is rather large and cumbersome to handle. The great care taken in explanation as opposed to mere recital of facts leads to a certain amount of repetition, but the main reason for the book's excessive length lies in the very rigid classification of its subject-matter. The book is in four parts: (1) introduction; (2) cells, intercellular substances and fluids; (3) the four primary tissues and their subdivisions; (4) the Histology of the systems. The introduction on what Histology is and how it is studied could hardly be better done, but if the student is to read the rest of the book in the sequence presented, his task will not be easy. The success of a book of this kind in the medical course depends very much on bringing the student as soon as possible into contact with the major systems of the body and not expecting him to carry forward too much introductory information just because it fits conveniently into a given classification. Apart from the introduction, almost 350 pages of this book are spent before the major systems of the body are discussed. It is difficult, for instance, to see what advantage is gained by treating cell structure in considerable detail at the beginning when much of it has to be repeated as soon as these details become significant for the description of a particular tissue or organ. A short chapter summarizing cellular details would be much more appropriately placed at the end of the book.

The author has been very concerned 'to make Histology more readily understood and, in particular, more easily remembered'. Does his classification and extended treatment of the four primary tissues help in these respects? It excludes the cells of the blood, for instance, so that we find them divorced from the haemopoietic tissues by over 100 pages. Indeed, another 100 pages has to be read before the circulatory system is even commenced.

Some will find Part 3 of the book quite irksome because much of this section robs the later chapters of what is essential for their general design. Why should bone and cartilage be placed amongst the tissues if the joints must go at the end of the book to be discussed as a system of articulations? This is not to say that classification of tissues should be abandoned. It can be mentioned quite briefly for what it is worth in an introductory chapter. If given overemphasis, classification interferes too drastically with the overall picture of the different regions of the body. After all, one does not find text-books of Pathology dealing in strict order with the Pathology of the cell, of epithelium and the rest of the tissues, before the organs and systems are allowed to come into the story.

The author is obviously rather worried about the lamentable state of histological terminology and tries to make it more palatable by giving Latin and Greek derivations, or by suggesting more logical terms. Thus it is obviously better to talk about acidophil leucocytes than to call these cells eosinophils, but common usage, we fear, will override even an International agreement to alter such terms. Perhaps it is better not to draw the student into controversies of this kind. At least the author should be consistent and not propose the term alveolus on page 178 for *all* non-tubular glands and then abandon it for the term acinus when describing the pancreas. A similar inconsistency is to be found in the labelling and legend of figure 417.

The language of this book will not appeal to all who are accustomed to reading the more impersonal style of conventional text-books. The aim of the author has been to talk to the student as he would in the practical laboratory rather than in the lecture theatre. Just occasionally his reasoning recalls the more elementary propositions of Euclid and one begins to doubt whether the medical student needs to be talked to in quite such a simple fashion, and whether indeed the wealth of similes, metaphors and everyday allusions always make their mark as intended. The following quotation might be used in fact to test a student's tolerance of the author's approach—'The areolar tissue of rodents contains a great many cells, hence areolar tissue obtained from a rat reveals large numbers of them.'

Taking the book as a whole, however, there is little important information which the author has not managed to include in this 2nd edition, and the reference lists at the end of each chapter, though almost exclusively in English, are more extensive and varied than usual. Perhaps the best chapters are on the skeleton, skin, lungs, liver and kidney, together with a much fuller treatment of intercellular substances and tissue fluid than can be found elsewhere. The illustrations are mainly photomicrographs supplemented by simple line drawings which are very clearly labelled and easily understood.

In conclusion, this is a book of outstanding merit which cannot fail to have a long and lasting influence on its subject: the more so if the author can be persuaded in future editions to fuse a chapter here and there, and not be too concerned with the foundations and plan of his original building.

K. C. RICHARDSON

*McFadyean's Osteology and Arthrology of the Domesticated Animals.* By H. V. HUGHES and J. W. DRANSFIELD, 4th ed. (Pp. vii+288; 205 line drawings; 21s.) London: Baillière, Tindall and Cox Ltd. 1953.

Towards the end of the last century, three years after the publication of his dissection guide of the horse in 1884, John McFadyean produced his book on *Osteology and Arthrology of the Domesticated Animals*. His translation shortly afterwards to the field of comparative pathology, a loss to veterinary anatomy mitigated to a considerable extent by the rise of another Edinburgh comparative anatomist Charnock Bradley, prevented further endeavours; but he did produce new editions of the 'Part I'.

In this latest edition, the first since McFadyean's death, Hughes and Dransfield have naturally adhered to the original method of presentation, accepting the legacy that descriptive anatomy of parts of the body may leave something to be desired and that it is difficult to adopt a standard nomenclature. The original illustrations have been redrawn and some fifty added. The classification of joints is more in keeping with functional considerations and throughout there is more information on development of bones.

C. W. OTTAWAY



BOOKS RECEIVED

*The Conception of Disease, its History, its Versions and its Nature.* By Walter Riese. (pp. 120; \$3.75.) New York, Philosophical Library.

*Proceedings of the Society for the Study of Fertility*, Number IV, London Conference, 1952. Cambridge, W. Heffer and Sons, Ltd.

*Manual of Comparative Anatomy*, second edition. By Osmond P. Breland. (xi + 256; \$4.50.) New York, McGraw-Hill Book Company, Inc.



# A QUANTITATIVE STUDY OF THE EFFECTS OF COMPOUND E, COMPOUND F, AND COMPOUND A, UPON THE BONE MARROW OF THE GUINEA-PIG

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## INTRODUCTION

The present work is a continuation of earlier attempts to study changes in the bone marrow by quantitative methods (Yoffey & Parnell, 1944; Yoffey, Metcalf, Herdan & Nairn, 1951; Hudson, Herdan & Yoffey, 1952). In a previous communication (Hudson *et al.* 1952) there were described the effects upon the bone marrow of daily injections of ACTH given for 7 successive days. It was thought possible that ACTH might act, either directly or indirectly, as a marrow stimulant, for it appeared to increase the total absolute count of nucleated marrow cells. The effect was evident both in the myeloid and in the erythroid cells, though more so in the latter.

However, in these experiments ACTH was administered to only nine animals. Since the result, if true, might be of fundamental importance in haematological work, it seemed desirable to try and establish the myeloplastic action of ACTH on a firmer foundation, and it was at first decided to repeat on a larger scale the experiments which had already been performed. On further reflexion, however, it was thought that ACTH might introduce too many variables, e.g. the uniformity of the ACTH used, the possibility of contamination, doubts as to whether any effects obtained might be due to ACTH itself or to suprarenal steroids, uncertainty whether the suprarenal cortex itself would always respond in the same way to ACTH stimulation, or whether a response by the output of several steroids would confuse the result unnecessarily. For reasons such as these it was finally thought preferable to use individual steroid hormones, compound E (cortisone), compound F (hydrocortisone) and compound A, although even with these there is always the possibility that any results obtained may be due not to the action of the particular steroid used, but to one or other of its intermediate compounds, or the induced metabolic effects.

## MATERIAL AND TECHNIQUE

### *Animals*

The work was done throughout on normal male guinea-pigs, of the Mill Hill strain originated by Dunklin and Hartley. Healthy animals about 2-3 months old were obtained from the University of Bristol Veterinary Field Station at Langford, and were kept in individual cages where they were carefully observed for 14 days before use.

\* The expense of this research has been defrayed by a generous grant from Messrs Reckitt and Colman Ltd., Hull. The cost of the illustrations has been met in part by a grant from the Colston Research Society.



*Compounds injected*

The experimental animals were given intraperitoneal injections of compound E, compound F or compound A suspended in a special medium. The greater part of the material used was supplied to us through the courtesy of Dr J. E. Garber of the Research and Development Division of Messrs Merck and Co., Rahway, New Jersey. Additional supplies of compound E and compound F, also prepared by Messrs Merck in an identical manner, were obtained from the Medical Research Council. For compound A we are indebted to Dr Choh Hao Li, of the University of California. The control animals were given daily injections of the suspending medium alone, consisting of: sodium chloride, 0.9 g.; benzyl alcohol, 0.9 ml.; polysorbate USP (Tween 80—Atlas Powder Co.), 0.4 g.; sodium carboxymethylcellulose (low viscosity), 0.4 g.; distilled water to 100.0 ml.

All the steroid compounds were given in single doses of 5 mg. daily for 7 days.

Altogether, seventy-seven experiments were performed, of which thirty were controls, twenty-nine were given compound E, ten compound A, and eight compound F. The compound E experiments were performed by three different groups of observers (groups I–III), each being responsible for ten controls and ten compound E animals, except that in one instance a compound E experiment was so obviously at variance with the entire series that it was discarded.

*Experimental procedure*

Animals were weighed at the beginning and end of the experimental period, as also were the two adrenal glands when the animals were killed. Peripheral blood, obtained from an ear vein, was examined at the beginning and end of each experiment; absolute counts were made of the total red and white cells and reticulocytes, as well as differential counts of the white cells. No special method, however, was used for the eosinophil counts. Haemoglobin was estimated by the Sahli method.

At the termination of each experiment, the second blood count was performed, after which under ether anaesthesia the abdomen was opened and the animal bled as freely as possible by cutting the lower end of the abdominal aorta. The blood was collected by a funnel into 15 ml. tubes, which were centrifuged immediately at 3500 r.p.m. in order to obtain serum.

Apart from obtaining serum, it appeared desirable, from the point of view of quantitative examination of marrow elements, to drain away from the marrow as much blood as possible, and it was thought that exsanguination might help considerably towards this end.

A small corked glass tube, between  $1\frac{1}{2}$  and 2 in. long, internal diameter about  $\frac{4}{16} - \frac{5}{16}$  in., is first weighed, then about half filled with autogenous serum, and weighed again. A humerus is then removed and cleaned, its two ends cut off, and the marrow ejected by a blower into the serum-containing tube which is then weighed for a third time. In this way one obtains a known weight of marrow suspended in a known weight of serum. The tube is then shaken in a mechanical shaker of about 6 in. amplitude for 2 min.—occasionally a little longer, if needed—at 400 times per minute. In the majority of cases the marrow disintegrates so that a uniform suspension is obtained, from which counts can be made in the usual manner, and also

dry smears can be prepared and stained. Immediately after shaking the suspension may be somewhat frothy, but the froth soon disappears. Before making a count or smear the tube is shaken by hand, or smartly tapped a few times. If this is not done the larger cells sink to the bottom of the tube, and only the smaller ones are obtained.

#### *Specific gravity*

If the specific gravity of both serum and bone marrow were precisely 1.0, the weights could be used to calculate the dilution. However, this is not the case, and so in each series there were calculated conversion factors, based upon specific gravity estimation both of bone marrow and serum. For the maximum degree of accuracy specific gravity determinations should perhaps have been made in each individual experiment. But in view of the large numbers of observations required in each case this was not found feasible; hence in each group of experiments a conversion factor calculated from seven or eight experiments was applied to the remainder of the group. The actual specific gravity estimation was done by a modified pycnometric technique.

#### *Dilution of marrow with residual blood*

In the absolute counts a modified Toisson's fluid, without acetic acid, was employed. This enabled absolute counts of both red and white cells to be made. The number of mature red cells in the bone marrow was taken as an indication of the maximum possible contamination with residual blood. The average red cell content in the entire series was approximately 700,000 per cu.mm. of marrow, while the red cells in the circulating blood were approximately 6,000,000 per cu.mm. It will be noted that the figure for marrow erythrocytes is considerably lower than in the previous paper (Hudson *et al.* 1952). This is possibly due to the fact that cutting the abdominal aorta is a more effective means of exsanguination than that formerly employed.

If all the red cells present in the marrow were in fact derived from residual blood, and not newly formed erythrocytes about to be discharged into the circulation, the marrow cells would be diluted with blood by about 1:9 and the calculated absolute counts would be proportionately below their true level. It should, however, be noted that the average marrow reticulocyte count is of the order of 300,000 per cu.mm.; these presumably have just been formed in the marrow and are awaiting discharge into the circulation, so that the marrow red cell count due to residual blood would be about 400,000 per cu.mm.

#### STAINED SMEARS

Dry smears were prepared on standard microscope slides and stained with Wright's stain, or MacNeal's tetrachrome, which was found particularly useful for the differentiation of cell granules. The preparation of the smears presents some difficulty, for if they are made slowly, the larger cells accumulate at the edges. If, on the other hand, the smears are made quickly, there is an even distribution of cells, but they are rounded off and not sufficiently spread out to facilitate the accurate observation of cytoplasmic and nuclear detail for purposes of cell identification. Furthermore,

in thin smears there is an increased number of damaged cells, which even in successful preparations can reach quite an appreciable figure (Table 2).

Accordingly, in each experiment fifteen to twenty marrow smears were made at different speeds, and then quickly examined unstained; a few slides with good distribution and relatively few damaged cells were then selected and stained. It was thought at one time that a Perspex spreader ( $\frac{3}{4}$  in. wide) seemed to produce better smears on the whole, with less damage to the cells, than the ordinary glass type; but while this was undoubtedly true on occasion, it is by no means a constant phenomenon. Counts were made at right angles to the long axis of the smears, as an additional safeguard against minor errors of distribution, and as a rule 1000 cells were counted.

#### SUPRAVITAL PREPARATIONS

In an attempt to throw light on the nature of the damaged cells, the marrow was studied in a number of cases by the supravital technique. After a number of trials the aqueous method was adopted in preference to the alcoholic, since it was found to give more uniformly reliable results. One or two drops of marrow were mixed with an equal quantity of dilute stain in a small ignition tube. The stain was freshly prepared by mixing solutions of neutral red (1/10 % in Ringer-Locke) and Janus green (1/10 % in Ringer-Locke) in proportions varying from 2:1 to 1:1 to 1:2 according to the concentration of cells in the marrow suspension. The mixture was gently shaken for 5–10 min. at room temperature, then left to stand in an incubator at 37° C. for 30 min. A drop of this mixture was then placed on a slide, and the cover-slip sealed with wax to prevent evaporation; the preparation was examined under oil immersion as rapidly as possible, a minimum of 500 cells being counted.

#### *Teased preparations*

In each animal pieces of abdominal and cervical lymph glands and thymus were teased in autogenous serum, and smears made and stained, in order to have undoubted lymphocytes for reference. This was important since one of the cells about whose identification we were particularly concerned was the small lymphocyte. It was also found that if fragments of bone marrow were similarly treated, the reticulum cells were damaged considerably less than in the standard preparations which were shaken for 2 min. Although unfortunately these teased preparations could not be used for quantitative purposes, as they contained numerous cell clumps, they gave valuable morphological information.

#### THE CLASSIFICATION OF CELLS

##### *(1) Supravital preparations*

In the supravital preparations, cell identification was based on the description given by Cunningham, Sabin & Doan (1925), and Doan, Cunningham & Sabin (1925). In general, it was found that there was fair agreement between these descriptions and the appearances seen in our preparations. There was one important exception, however, for in our supravital preparations there were a number of large cells with spherical nuclei, and cytoplasm containing only a few large mitochondria. These are not the primitive cells of Cunningham *et al.* (1925), though they may possibly



correspond to the reticulum cells (Pl. 1, figs. 1-7) of the stained smears. However, as this is not certain, the cells have been placed in the unclassified group.

Lymphocytes were identified as such only if they resembled similar cells obtained from a teased lymph gland of the same animal. The nuclei, frequently indented, are typically large in relation to the cytoplasm, which forms a thin rim containing mitochondria and a few small red vacuoles.

## (2) *Stained smears*

The reticulum cells, for the reasons already mentioned, could not be studied quantitatively. Apart from these, the classification of cells was into four main groups, myeloid, erythroid, monocytoid and lymphocytic. In addition, there was a large group of damaged cells, a varying number of unclassified cells, and a few miscellaneous cells such as plasma cells and macrophages.

Reticulum cells present a number of difficulties, for the term, though widely used in haematological literature, is usually somewhat loosely defined. The most obvious use of the term is for cells which are actually arranged in a reticulum, and the tearing away of these cells from such a reticulum might account for the fact that they are so readily damaged. It was in this sense apparently that Cunningham *et al.* (1925) used the term (or rather 'reticular'). Pl. 1, figs. 1-7, illustrate what are described as reticulum cells in the present investigation, but we are not altogether satisfied that they are in fact part of a network of cells.

These reticulum cells seem to correspond to what Rohr (1949) calls large reticulum cells (his small reticulum cells being almost impossible to distinguish from the small lymphocyte), and they also in some ways resemble what Ferrata (1918) has termed haemohistoblasts, more especially those found under certain pathological conditions (1918, Vol. II, Tav. xv).

The reticulum cells consist of pale grey-blue cytoplasm, in which there is a characteristic leptochromatic nucleus, with a clearly defined border, chromatin strands of uniform and equal thickness (isochromatic), and several nucleoli. These cells form an appreciable, possibly even the major, part of the damaged cells; and they may often be recognized, even when the cytoplasm is all gone and the nucleus is swollen and disrupted, by the persistence of the more resistant nucleoli. It is not difficult to find transition stages between these cells and the typical myeloblasts, and usually as the cytoplasm becomes more basophilic the nuclear chromatin loses its sharply defined network, while the nucleoli tend to become less conspicuous and disappear.

Lymphocytes (Pl. 1, figs. 8-14) were classified on the basis of their morphology, and as in the supravital preparations, cells in stained smears of lymph glands (Pl. 1, figs. 12-14) were carefully examined in all experiments before performing the differential count of the bone marrow and the identification of marrow lymphocytes (Pl. 1, figs. 8-11). The number of lymphocytes was smaller in the supravital preparations than in the stained smears (Table 3), and this was largely due to the difficulty of distinguishing between lymphocytes and monocytes in the supravital preparations. This is an old problem (see, for example, Cappell, 1929; Wiseman, 1931-2; Hall, 1938; Drinker & Yoffey, 1941), and since the significance of the neutral

red bodies has been so frequently questioned, the stained smear figures are likely to be more reliable.

It should be emphasized that practically all the marrow lymphocytes are small lymphocytes, though a very few were found of medium size (Pl. 1, fig. 10). The nucleus of medium lymphocytes tends to be more leptochromatic than that of the small, though some definite chromatin clumps are still present. No cell with a fully leptochromatic nucleus has been counted as a lymphocyte.

The granulocytes (Pl. 2, figs. 16-23) and the erythroid series (Pl. 2, fig. 24, and possibly fig. 15) call for little comment. The great majority of the myeloid cells belong to the neutrophil—or in the guinea-pig more accurately pseudo-eosinophil—group. Basophil myelocytes (Pl. 2, figs. 19-20) are few in number, but form a very striking group of cells. Though it is quite true that on occasion some of the basophil granules seem to have a quite distinct eosinophil staining component, nothing was seen to indicate the transformation of basophil into eosinophil myelocytes as described by Downey (1915), though very rarely we saw, in an otherwise typical eosinophil cell, one or two deeply basophilic granules.

Monocytes were seen developing from blast cells like those of the myeloid and erythroid series. The term 'metamonocyte' is used for the cell depicted in Pl. 1, fig. 11.

## RESULTS

### (1) *Specific gravity*

Table 1 gives the specific gravity of serum and bone marrow with the calculated conversion factors, in seven control guinea-pigs, seven treated with compound E, and eight with compound F. The specific gravity of the bone marrow has increased in both the compound E and the compound F experiments, the mean increase in the former case reaching the conventional significance level ( $t_{C-E}=2.432$ ,  $P<0.5$ ;  $t_{C-F}=1.74$ ,  $P>0.10$ ).\*

### (2) *Cellular changes*

#### (a) *Total count*

The compound E experiments, as has already been noted, were performed by three different groups. The total absolute count of nucleated cells showed a marked mean increase in group I ( $t_{C-E}=2.5$ ,  $P<0.025$ , the means rising from 1,689,900 per cu.mm. to 2,329,700), and group II ( $t_{C-E}=2.23$ ,  $P<0.05$ , the means rising from 1,685,000 to 2,131,600), whereas in group III there was actually a slight fall, not significant ( $t_{C-E}=0.19$ ,  $P>0.50$ , mean falling from 1,605,300 to 1,572,000). In the one group of compound F experiments there was also a mean rise in the total count ( $t_{C-E}=2.10$ ,  $P=0.05$ , means rising from 1,689,900 to 2,141,130). In the ten compound A experiments, the mean count was 1,438,200. But this may in part be due to the fact that one of the counts at 925,900 was well below the usual range and showed other abnormal features also. The discrepancy between group III and the other groups is one for which no explanation can be offered. The effect of the initial discrepancy in the absolute count is subsequently reflected to a large extent in the

\* In giving these 't' values, C=control series, A=compound A, E=compound E, F=compound F experiments,  $t$ =Student's  $t$ ,  $P$ =probability of a greater  $t$  value arising on pure chance.

absolute counts of the various cell groups. Table 2 gives the results of group I and illustrates the way in which the mean data of the different groups were arranged for the purposes of analysis.

Table 1. *Specific gravity of serum and bone marrow, with conversion factor, in seven control guinea-pigs, seven treated with compound E, and eight with compound F*

Serial no. of animal	Serum specific gravity	Bone marrow specific gravity	Conversion factor
Control series			
A7	1.0200	1.0284	1.0078
A9	1.0184	1.0313	1.0130
A11	1.0217	1.0341	1.0118
A13	1.0191	1.0242	1.0050
A15	1.0209	1.0174	0.9966
A17	1.0193	1.0468	1.0271
A19	1.0205	1.0363	1.0153
Arith. mean	1.0200	1.0312	1.0114
S.D.	0.001124	0.00870	0.00847
Compound E series			
A8	1.0248	1.0450	1.0195
A10	1.0253	1.0651	1.0389
A12	1.0252	1.0548	1.0292
A14	1.0370	1.0829	1.0442
A16	1.0207	1.0507	1.0294
A18	1.0200	1.0442	1.0238
A20	1.0225	1.0420	1.0191
Arith. mean	1.0249	1.0549	1.0320
S.D.	0.00526	0.01355	0.00836
Compound F series			
A21	1.0139	1.0620	1.0473
A22	1.0220	1.0240	1.0021
A23	1.0194	1.0442	1.0248
A24	1.0223	1.0500	1.0274
A25	1.0120	1.0743	1.0632
A26	1.0099	1.0396	1.0500
A27	1.0211	1.0420	1.0297
A28	1.0194	1.0560	1.0363
Arith. mean	1.0175	1.0490	1.0340
S.D.	0.00453	0.0391	0.0180

### (b) Erythroid cells

The erythroid cells show a mean rise in all three groups (group I mean rises from 358,400 to 659,930,  $t_{C-E}=3.82$ ,  $P<0.01$ ; group II mean rises from 368,720 to 672,000,  $t_{C-E}=3.58$ ,  $P<0.01$ ; group III mean rises slightly from 376,000 to 411,600,  $t_{C-E}=0.53$ ,  $P>0.50$ ; compound F mean rises from 358,440 to 522,080,  $t_{C-E}=2.40$ ,  $P$  approx. 0.025). If one combines all these results, then  $t_{C-E}=2.9$ ,  $P<0.01$ , and the mean rises from 367,700 to 561,000. It will be noted that even in group III, where the increase does not reach the conventional significance level, the trend is, nevertheless, in the same direction. The supravital counts in this latter group showed a somewhat more marked rise, the mean going up from 324,700 to 470,000, but it is difficult to make any direct comparison between these and the counts in the stained preparations.



(c) *Myeloid cells*

Though the myeloid cells have increased in all compound E experiments, the mean increase is not as marked as in the case of the erythroid cells. The figures are: group I, 602,330-745,070,  $t_{c-E}=1.57$ ,  $P>0.10$ ; group II, 579,230-765,600,  $t_{c-E}=1.85$ ,  $0.05 < P < 0.1$ ; group III, 608,600-625,600,  $t_{c-E}=0.22$ ,  $P>0.50$ .

Table 2. *Absolute counts per cu.mm. (group I) of the main groups of nucleated cells in the bone marrow of ten control guinea-pigs, ten after administration of compound E (5 mg. daily for 7 days) and ei after administration of compound F (5 mg. daily for 7 days). (Eosinophils are given in separate column, and also included among 'Myeloid cells').*

No. of exp.	Erythroid	Myeloid	Eosinophils	Lymphocytes	Monocytes	Damaged	Unclassified	Total abs. count of nucleated cells	Myeloid:erythroid ratio
Control									
A 1	185,000	476,000	87,050	272,000	142,800	234,000	31,800	1,360,000	2.55
A 3	166,500	383,200	38,800	193,900	36,680	221,000	41,890	1,048,000	2.38
A 5	322,210	472,200	99,000	432,000	95,350	182,000	79,780	1,596,000	1.44
A 7	234,000	418,800	98,100	259,800	62,080	276,200	55,220	1,381,000	1.87
A 9	430,800	831,200	72,400	386,800	124,800	231,300	61,400	2,079,000	1.99
A 11	524,000	551,800	41,800	349,000	115,600	350,600	35,680	1,995,000	1.07
A 13	415,100	609,312	53,661	320,235	100,398	252,726	27,696	1,731,000	1.47
A 15	457,090	846,060	108,900	247,000	114,760	243,110	29,174	1,945,000	1.87
A 17	255,500	582,100	59,990	260,000	75,420	180,400	34,920	1,396,000	2.22
A 19	594,200	820,000	71,100	478,800	65,800	226,800	73,500	2,368,000	1.38
Mean	358,440	599,067	73,076	319,954	93,369	239,814	47,106	1,689,900	1.87
Compound E (acetate)									
A 2	1,015,000	796,000	205,000	468,000	247,900	355,800	94,900	3,060,000	0.77
A 4	416,500	556,000	66,000	333,100	86,900	170,600	46,710	1,610,000	1.38
A 6	566,800	566,800	115,700	375,000	119,700	309,000	45,970	1,995,000	1.07
A 8	554,000	610,000	100,500	398,000	75,800	193,600	66,300	1,898,000	1.11
A 10	990,000	1,162,000	147,600	534,900	115,800	570,000	70,400	3,510,000	1.11
A 12	490,100	671,800	101,260	451,900	68,400	268,100	46,300	2,010,000	1.38
A 14	713,800	1,130,000	109,300	474,900	150,500	330,800	71,986	2,876,000	1.57
A 16	515,500	520,000	88,080	278,000	110,200	217,000	47,490	1,695,000	1.07
A 18	635,920	686,120	83,234	255,800	79,180	310,600	44,675	2,030,000	1.07
A 20	700,800	752,900	36,580	528,600	85,000	392,000	128,000	2,613,000	1.07
Mean	659,842	645,162	105,325	409,820	113,938	311,750	66,271	2,329,700	1.11
Compound F (free alcohol)									
A 21	544,750	450,400	34,506	490,350	54,475	207,010	59,923	1,816,000	0.88
A 22	371,500	560,000	91,550	448,300	73,240	291,000	65,880	1,831,000	1.57
A 23	302,500	446,900	58,390	259,600	57,180	174,100	54,880	1,298,000	1.44
A 24	486,000	706,000	87,220	436,000	122,500	228,200	72,310	2,078,000	1.44
A 25	748,000	950,800	55,600	581,800	210,200	423,200	151,500	3,090,000	1.22
A 26	603,260	853,890	155,780	340,650	96,983	449,480	10,643	2,460,000	1.44
A 27	511,550	771,610	133,350	378,180	109,270	330,140	65,584	2,186,000	1.57
A 28	609,120	839,080	105,420	424,220	97,162	317,620	61,616	2,370,000	1.38
Mean	522,150	697,440	90,157	419,890	102,501	302,594	67,796	2,141,000	1.38

(d) *Myeloid:erythroid ratio*

The mean ratio dropped markedly in group I ( $1.83-1.14$ ,  $t_{c-E}=4.2$ ,  $P<0.01$ ); not so markedly in group II ( $1.661-1.275$ ,  $t_{c-E}=2.17$ ,  $P<0.05$ ); and only slightly in group III ( $1.616-1.520$ ,  $t_{c-E}=0.22$ ,  $P>0.50$ ). In all three groups, though the total count of myeloid cells had risen, that of the erythroid cells had risen rather more, and hence the myeloid:erythroid ratio has fallen.

(e) *Lymphocytes*

The marrow lymphocytes have shown no consistent trend. They rose significantly in group I ( $t_{c-E}=2.12$ ,  $P<0.05$ ), and slightly in group II ( $t_{c-E}=0.43$ ,  $P>0.50$ ), whereas they fell in group III ( $t_{c-E}=0.98$ ,  $0.10<P<0.50$ ). Combining all the compound E experiments, the mean lymphocyte count rises from 322,000 to 338,420 ( $t_{c-E}=0.38$ ,  $P>0.50$ ).

Table 3. A comparison of the supravital and stained smear techniques. Absolute counts per cu.mm. of the main cell groups in the bone marrow of ten guinea-pigs after the administration of compound A (5 mg. daily for 7 days)

	Total erythroid	Total myeloid	*Eosinophils	Total lymphocyte	Mono-cyte	Damaged	Unclassified	Others†	Total nucleated cells	M:E ratio
					Supravital					
	413,700	600,100	35,000	113,600	192,300	—	113,600	23,300	1,456,600	1.45
	229,600	374,000	16,600	114,800	74,100	—	105,500	27,900	925,900	1.63
	278,800	728,600	17,900	137,900	152,900	—	80,900	120,000	1,499,100	2.62
	341,000	721,600	53,000	241,600	188,700	—	66,200	95,900	1,655,000	2.12
	262,200	957,800	117,700	90,800	141,200	—	77,300	151,100	1,680,400	3.66
	301,000	804,800	72,000	266,000	180,000	—	32,600	52,100	1,635,700	2.68
	184,800	639,400	102,400	122,400	87,400	5,000	85,000	124,800	1,248,800	3.46
	492,000	755,200	84,200	188,000	58,400	9,800	77,800	39,600	1,620,800	1.55
	367,200	597,000	35,000	189,400	113,600	—	67,000	123,100	1,457,300	1.63
	214,000	490,600	31,200	151,400	141,800	5,000	122,600	77,000	1,202,400	2.29
ages	308,400	667,000	56,500	161,600	133,000	2,000	82,800	83,400	1,438,200	2.31
					Stained smears					
	332,100	606,000	55,000	135,500	182,100	150,000	27,700	23,200	1,456,600	1.83
	199,100	369,400	27,700	168,500	43,500	97,300	31,500	16,600	925,900	1.86
	379,300	500,700	44,900	319,300	71,900	131,500	34,500	61,900	1,499,100	1.36
	448,500	680,200	115,800	147,300	142,300	177,100	15,000	44,600	1,655,000	1.52
	327,700	784,700	89,100	201,700	92,400	201,700	16,800	55,400	1,680,400	2.40
	340,200	494,000	47,400	616,700	40,900	72,000	37,600	34,300	1,635,700	1.45
	332,200	422,100	53,700	144,900	40,000	229,800	15,000	64,800	1,248,800	1.27
	518,700	656,400	27,600	273,900	22,700	71,200	21,000	56,900	1,620,800	1.27
	304,600	587,300	29,100	347,000	58,300	94,700	18,900	46,500	1,457,300	1.93
	221,400	486,900	20,400	282,700	28,900	11,800	30,100	40,600	1,202,400	2.20
ages	340,400	558,800	51,100	263,700	72,300	133,800	24,800	44,400	1,438,200	1.71

\* Eosinophils are also included in the column headed 'total myeloid'.

† 'Others' comprises a small group of miscellaneous cells such as plasma cells, macrophages, reticulum cells.

(f) *Eosinophils*

The eosinophils do not show a constant trend. The means and 't' values for the three groups are group I, 73,080–105,320,  $t_{c-E}=1.92$ ,  $0.05<P<0.10$ ; group II, 61,330–93,580,  $t_{c-E}=1.42$ ,  $0.10<P<0.50$ ; group III 50,400–56,500, ( $t_{c-E}=0.67$ ,  $P>0.50$ ). It will be seen that on the whole the tendency is for the marrow eosinophils to rise, but that nowhere does the rise attain the conventional significance level.

(g) *Reticulocytes*

There was no marked change in the marrow reticulocytes, though here, too, the mean values seemed to be rising in the experimental animals. Group I, 224,000–292,000,  $t_{c-E}=1.35$ ,  $0.10<P<0.50$ ; group II, 279,030–394,590,  $t_{c-E}=1.65$ ,  $P=0.10$  approx.; group III, 277,780–325,300,  $t_{c-E}=0.40$ ,  $P>0.50$ .

## DISCUSSION

*Dosage and sensitivity*

In comparing the present results with those of other workers, it is important to bear a number of factors in mind. One factor is obviously that of dosage, differences in which can probably contribute very materially to differences in results. A second factor is that of sensitivity. Thus it is generally held that the guinea-pig is somewhat insensitive to cortisone. Species differences of sensitivity may be due not only to the substance administered, but also to variations in the metabolism of these substances and the intermediate products of that metabolism.

*Specific gravity*

The increase in specific gravity is in all probability attributable to an increase in the cellularity of the marrow, with a consequent diminution in its fat content. Mechanik (1926) has shown that fatty marrow has a specific gravity slightly below 1.0, red marrow somewhat above 1.0. In the present experiments similar considerations probably apply, except that all the marrows were 'red' to varying degrees. If this interpretation of the specific gravity changes is correct, it could be regarded as confirmatory of the increase in absolute count obtained in groups I and II. In group III, unfortunately, no specific gravity determinations were made.

However, though it may well be the case that the rise in specific gravity is probably associated with increased cellularity of the marrow, it should be noted that, in the twenty-two experiments in which the specific gravity of the marrow was actually determined, it was not found possible to establish a significant statistical correlation between the specific gravity and the total count of nucleated cells.

*Lymphocytes**No significant difference between control and experimental series*

As far as lymphocytes are concerned, there is no marked difference between the control and the experimental series. This is equally true whether the bone marrow is examined in stained smears or in supravital preparations. The figures for marrow lymphocytes are of the same order as those in normal guinea-pigs employed in previous experiments, and the injection of the suspending medium in the control experiments does not appear to have materially altered the number of these cells.

*Large number of lymphocytes in marrow*

Although, therefore, none of the steroid compounds employed seems to have had any obvious effect upon the marrow lymphocytes, the present—and much longer—series of experiments seems to confirm quite unequivocally the previous findings (Yoffey & Parnell, 1944) concerning the large numbers of lymphocytes in the bone marrow. As compared with the earlier—and technically much less satisfactory—results in the rabbit, the present series gives an average marrow lymphocyte content of 322,000 per cu.mm. This is about six times the concentration first noted in the rabbit, and when one compares it with a count of about 4000–5000 lymphocytes per cu.mm. of blood, it becomes clear that the high concentration of marrow lympho-



cytes completely rules out the possibility that their presence is due to blood contamination.

In an earlier paper (Yoffey & Parnell, 1944) attempts were made to compare the total blood and bone marrow lymphocytes, and correlate them with known data concerning thoracic duct lymphocyte output. It was then concluded that even on the basis of 60,000 lymphocytes per cu.mm., the number of lymphocytes in the marrow was sufficient to account for those daily leaving the blood. In the case of the guinea-pig, the present studies seem to make it clear that the number of lymphocytes in the marrow is well in excess of what would be needed to account for those disappearing from the blood.

#### *Marrow lymphocytes occur as scattered cells*

It is noteworthy that lymphocytes in the normal guinea-pig marrow occur as scattered single cells. It cannot be emphasized too strongly that in *normal* marrow one rarely sees even small accumulations of lymphocytes, still less organized follicles with or without germinal centres. The literature contains frequent references to the occurrence of lymphoid nodules in human marrow, and the significance of these has been previously discussed (Drinker & Yoffey, 1941; Jordan, 1935). But it is clear from the present material that, in the normal guinea-pig, lymphoid follicles are not a regular constituent of bone marrow, for in not a single one of the seventy-seven animals examined were any follicles found in occasional sections of the marrow. It is further to be noted that mitoses in marrow lymphocytes are singularly infrequent; we have not seen any, nor apparently have other workers seen more than a very few (cf. Leitner, 1949). The bone marrow, therefore, is not to be regarded as a region of lymphocyte production, unless one postulates a constant heteroplastic formation of lymphocytes from reticulum cell or myeloblast. Unlike lymphoid tissue, however (see, for example, Downey & Weidenreich, 1912), the heteroplastic formation of lymphocytes in the bone marrow is difficult to observe.

In fact, despite the large number of lymphocytes occurring in the bone marrow, it is infrequent to see evidence of transition forms which would indicate either the origin of lymphocytes from, or their transformation into, other cells. If such transformation occurs one can only assume that it must take place quite rapidly, so that as a rule it is not detected.

#### *No lymphoclastic action of 11-oxysteroids*

In the twenty-nine compound E marrows the average concentration of lymphocytes at 338,420 per cu.mm. showed a slight but insignificant increase over the normal. It is evident, then, that if there has been no significant increase in marrow lymphocytes after the administration of compound E (and similarly with compounds F and A), there is equally no obvious decrease. The bone marrow data do not therefore support the concept that the 11-oxysteroids possess a lymphoclastic action.

#### *The identity of the lymphocyte*

One further point needs perhaps to be made concerning the lymphocytes. They have been identified as such in the present experiments on morphological grounds; and after repeated comparison of cells in bone marrow and lymph node smears,

the evidence in favour of this identification appears sound. Other views, however, have been expressed. For example, Sabin, Miller, Smithburn, Thomas & Hummel (1936) examined the bone marrow of forty-nine young rabbits, and could not find a single lymphocyte. This finding is somewhat difficult to explain. Even in adult marrow it would be more than surprising that among many thousands of cells a few lymphocytes should not be found, if only as a result of blood contamination. In the marrow of young animals the presence of a fair number of lymphocytes would appear to amount to a certainty, and the fact that none was described suggests that the criteria employed in identification were faulty.

Sabin *et al.* (1936) must in fact be considered to have placed their lymphocytes in the same category as the 'primitive' cell, which they admitted (p. 115, *loc. cit.*) 'looks very much like the small lymphocyte' though it 'lacks certain signs of differentiation'. They emphasize that 'the so-called primitive cell occurs in the bone marrow diffusely scattered and not in the germ centre'. But this latter observation could, with equal validity, be adduced in support of the view that lymphocytes are continually passing into the marrow from the blood. Rohr (1949) does not completely deny the occurrence of small lymphocytes in the bone marrow, but considers that many of the cells which are so described are small reticulum cells, though he admits that the distinction between these cells and the small lymphocytes is one which may be extremely difficult to draw.

It seems to us that the evidence so far available definitely favours the view that the cells in question are small lymphocytes, and that they are continually migrating into the bone marrow from the blood. However, should this view prove to be erroneous, and should these cells belong to a different category, the quantitative data concerning their numbers would still possess great significance, and render it highly probable that they play an important part in the process of blood formation.

In ten compound A experiments an attempt was made to apply Sabin's own criteria, using the supravital technique for the identification of lymphocytes. Though it is true that in this instance (Table 3) there were fewer lymphocytes in the supravital preparations than in the stained smears, the former nevertheless gave the figure of 160,000 per cu.mm., which is still nearly three times as high as the original estimate of lymphocytes in rabbit marrow (Yoffey & Parnell, 1944).

#### *Reticulum cells*

In the compound A animals, granules were much more in evidence in the reticulum cells, which also appeared to be more numerous than in normal marrows, though for the reasons already given reliable quantitative data are not available. The granules in the reticulum cells are mainly azurophilic, and sometimes attain quite a large size (See Pl. 1, fig. 6). It would no doubt be quite possible to interpret these as phagocytic reticulo-endothelial cells. The literature contains conflicting views on the phagocytic nature of the early stem cells. Ferrata (1918: see his Tav. XX and XXI, Vol. 1) depicts as 'cellule emoistioblastiche' cells which all look like macrophages or monocytes. In his Tav. XXI the haemohistoblasts have ingested large amounts of lithium carmine. It is of interest, too, that the haemohistoblasts which he chooses for purposes of illustration are mainly taken from spleen, lymph gland,

or connective tissue, but not from bone marrow. Cunningham *et al.* (1925), on the other hand, depict their 'reticular' cell as one which is quite unspecialized and inert, incapable of phagocytic activity. (See also Pappenheim, 1919.)

#### *The myeloid:erythroid ratio*

On the whole the effect of compound E has been to increase the erythroid cell content of the marrow and probably the myeloid cells also, but the increase in the former has been greater, with the result that the M:E ratio has fallen. It is all the more interesting therefore to note that the effect of adrenalectomy appears to be the reverse. Thus Gordon, Piliero & Landau (1951), working with mice, found that following adrenalectomy, what they termed the E:M (erythroid:myeloid) ratio diminished, from 1.46 in the controls to 0.91 after 1 week and 0.73 after 2 weeks, but then started to rise somewhat, reaching 0.95 after 3 weeks, and 1.01 after 4 weeks. These changes in the marrow were accompanied by a mild anaemia. On the other hand, the administration of compound E (3 mg. daily for 14 days) appeared to restore the E:M ratio to near the normal level (1.29). This is somewhat difficult to reconcile with the finding that sections of bone marrow displayed 'a considerable reduction in total cellularity due to increased vacuolization'. The experiments just quoted seem to emphasize once again the problems of dosage and species. Gordon *et al.* (1951) gave their mice 3.0 mg. of compound E daily in order to prevent the development of a peripheral anaemia. Doses of 0.5 and 1.0 mg. were not effective, even though in relation to body weight they represent a much bigger dose than the 5 mg. per day given to the guinea-pigs in the present series of experiments.

Appearances suggesting degenerative changes in the marrow were also observed by Baker & Ingle in the rat (1948) after the administration of ACTH. Gordon *et al.* (1951) suggest that high doses of compound E—and presumably of ACTH also—induce an excessive deposition of fat, as previously noted also by Winter, Silber & Stoerk (1950). However, in the present experiments there was no sign whatever of increased deposition of fat in the bone marrow, and the dosage presumably is approximating more to the physiological levels.

Finally, as far as the bone marrow is concerned, the present findings seem to accord with the clinical conclusions of Wintrobe, Cartwright, Palmer, Kuhns & Samuels (1951) that '...the principal alterations in the blood seen in the diseases other than leukemia, were those of increased bone marrow activity'.

#### *Blood changes*

Few significant changes were observed in the peripheral blood. Lymphocytes showed no change of note in the compound E experiments, but in the compound A series there was an actual increase, the mean in ten experiments rising from 3890 to 5450 ( $t_{c-A}=2.11$ ,  $P=0.5$ ). It may be that one is dealing here with too small a series (10) of animals on which to base a really firm conclusion, but the result is so much at variance with what is usually thought to be the response of blood lymphocytes to 11-oxysteroids that it seemed worthy of note, and even of further investigation in a larger series. The blood eosinophils were not examined by a chamber technique, and no valid conclusions can be drawn from the ordinary method of counting.



There was also an increase in the blood reticulocytes in the twenty-nine compound E experiments, the mean count rising from 69,600 to 114,000,  $t_{C-E}=2.96$ ,  $P<0.01$ . Despite the fact that only 1000 cells were counted in each case (see, for example, Marcussen, 1939) this increase is probably a reflexion of increased activity in the erythroid cells of the bone marrow, and may be regarded as affording confirmatory evidence of the stimulating action of compound E upon erythropoiesis. The rise in the blood reticulocytes appears also to argue against the view put forward by Quittner *et al.* (1951), to the effect that cortisone acts by producing a block to the escape of cells from the bone marrow.

#### *The response to different steroids*

One of the more interesting general problems in connexion with the steroid hormones is that of the differences in action between a number of closely related substances. The view seems to be gaining ground that compound F is more potent than compound E. Recently, Hungerford, Reinhardt & Li (1952) have reported that 'at comparable dose levels hydrocortisone is demonstrably and significantly more effective in producing a thoracic duct lymphopenia than is cortisone'. However, as far as the bone marrow is concerned, the present experiments have afforded no evidence of such a difference. It is true that only eight experiments were performed with compound F (see Table 2), but it seems difficult to believe that had a marked difference really existed, these experiments would have given no indication of it.

Although no very clear-cut differences could be established between compounds E and F, the compound A experiments appeared to reveal two distinctive features, to both of which reference has already been made. In the bone marrow there was noticed an effect upon granulopoiesis; granule formation seemed to occur with marked frequency in reticulum cells, which matured to a myelocyte stage rapidly, with little sign of typical myeloblast formation. If this observation is correct, more prolonged administration of compound A should result in an increase in myeloid cells which can be measured quantitatively. It would be interesting at the same time to note whether the more prolonged administration of compound A gives rise to an enhanced lymphocytosis.

#### SUMMARY

1. In seventy-eight male guinea-pigs, 2-3 months old, the action upon the bone marrow of a number of steroid hormones has been studied by a quantitative technique.

2. The administration of compound E, in doses of 5 mg. per day for 7 days, appears to stimulate increased red cell formation in the bone marrow. There may be a concomitant increase in the myeloid cells, but this is not so marked, and thus there is a fall in the myeloid:erythroid ratio.

3. Compound A given to ten animals (5 mg. daily for 7 days) gave rise to a moderate but definite lymphocytosis in the blood, while in the marrow it seemed to stimulate granulocyte formation.

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## EXPLANATION OF PLATES

(Figs. 1-24. The figures are all untouched photomicrographs of stained smears, taken at a magnification of  $\times 1330$ , of guinea-pig bone marrow or lymph node.)

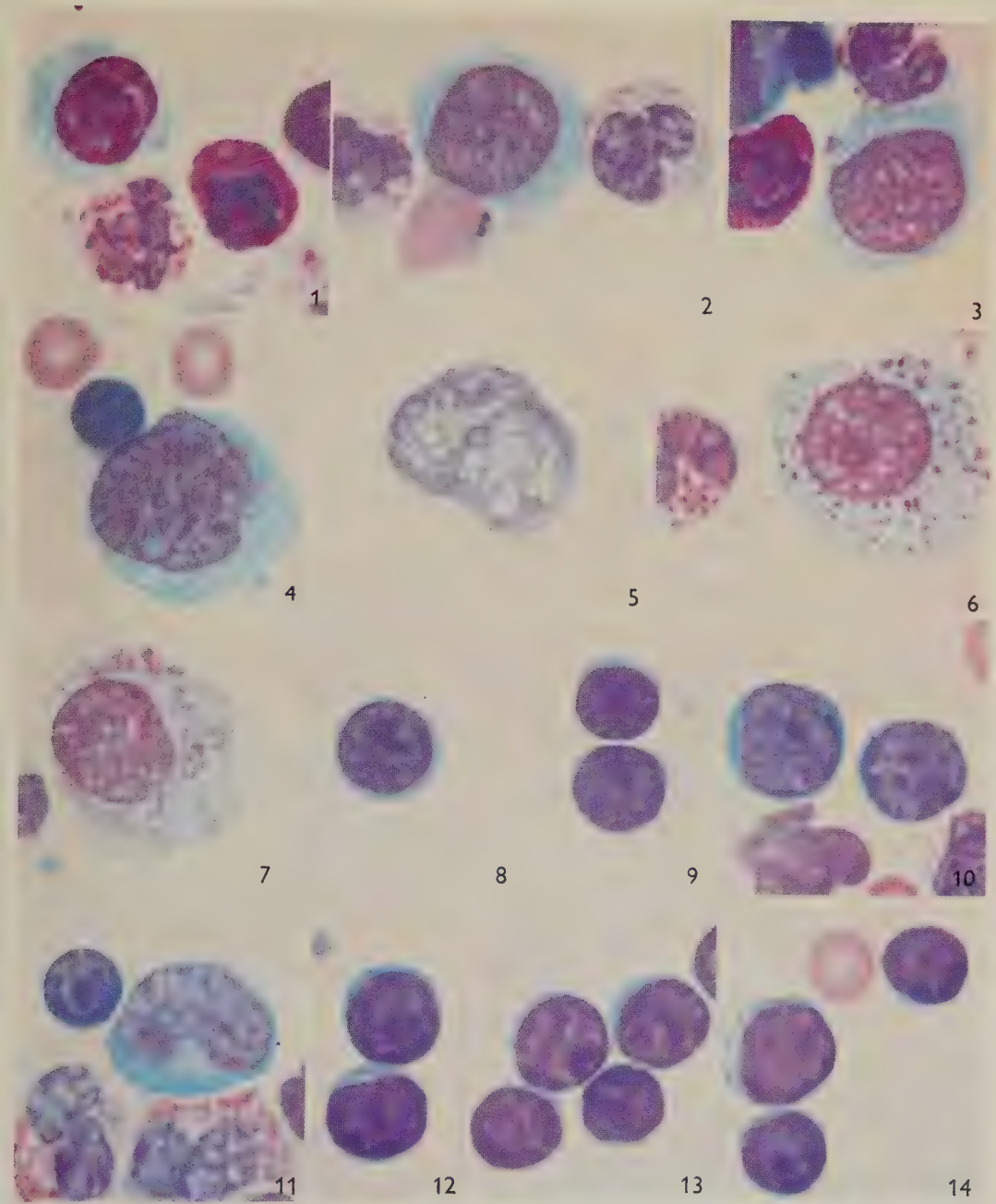
## PLATE 1

- Fig. 1. Reticulum cell, without granules, from the bone marrow of a guinea-pig which has been given compound A. Note blue-grey somewhat mottled cytoplasm, with irregular edge. Nucleus possesses sharp nuclear membrane, and several nucleoli.
- Fig. 2. Reticulum cell from bone marrow of a normal guinea-pig. A somewhat larger cell than no. 1.
- Fig. 3. Reticulum cell from bone marrow of normal guinea-pig. Cytoplasm showing more obvious signs of damage than figs. 1 and 2.
- Fig. 4. Reticulum cell—from bone marrow of normal guinea-pig—somewhat more damaged than fig. 3.
- Fig. 5. From bone marrow of normal guinea-pig. Still more advanced stage of reticulum cell damage. The nucleoli still persist.
- Fig. 6. From bone marrow of guinea-pig after treatment with compound A. Reticulum cell with granules, mainly azurophilic.
- Fig. 7. From bone marrow of normal guinea-pig. Later stage than fig. 6 of transformation of reticulum cell to granulocyte. This type of cell has much in common with Pappenheim's leucoblast (1919).
- Fig. 8. Small lymphocyte from bone marrow of normal guinea-pig. Typically pachychromatic nucleus, moderate cytoplasmic basophilia.
- Fig. 9. Two small lymphocytes from bone marrow of normal guinea-pig.
- Fig. 10. Small and medium lymphocyte from bone marrow of normal guinea-pig. The larger of the two cells has a somewhat more leptochromatic nucleus, and in some ways resembles a blast cell.
- Fig. 11. Small lymphocyte and metamonocyte from bone marrow of normal guinea-pig.
- Fig. 12. Two small lymphocytes from lymph gland (teased) of normal guinea-pig.
- Fig. 13. Group of small lymphocytes from teased lymph gland of normal guinea-pig.
- Fig. 14. One medium lymphocyte and two small lymphocytes from teased lymph gland of normal guinea-pig. Compare the medium lymphocyte with the similar cell in fig. 10.

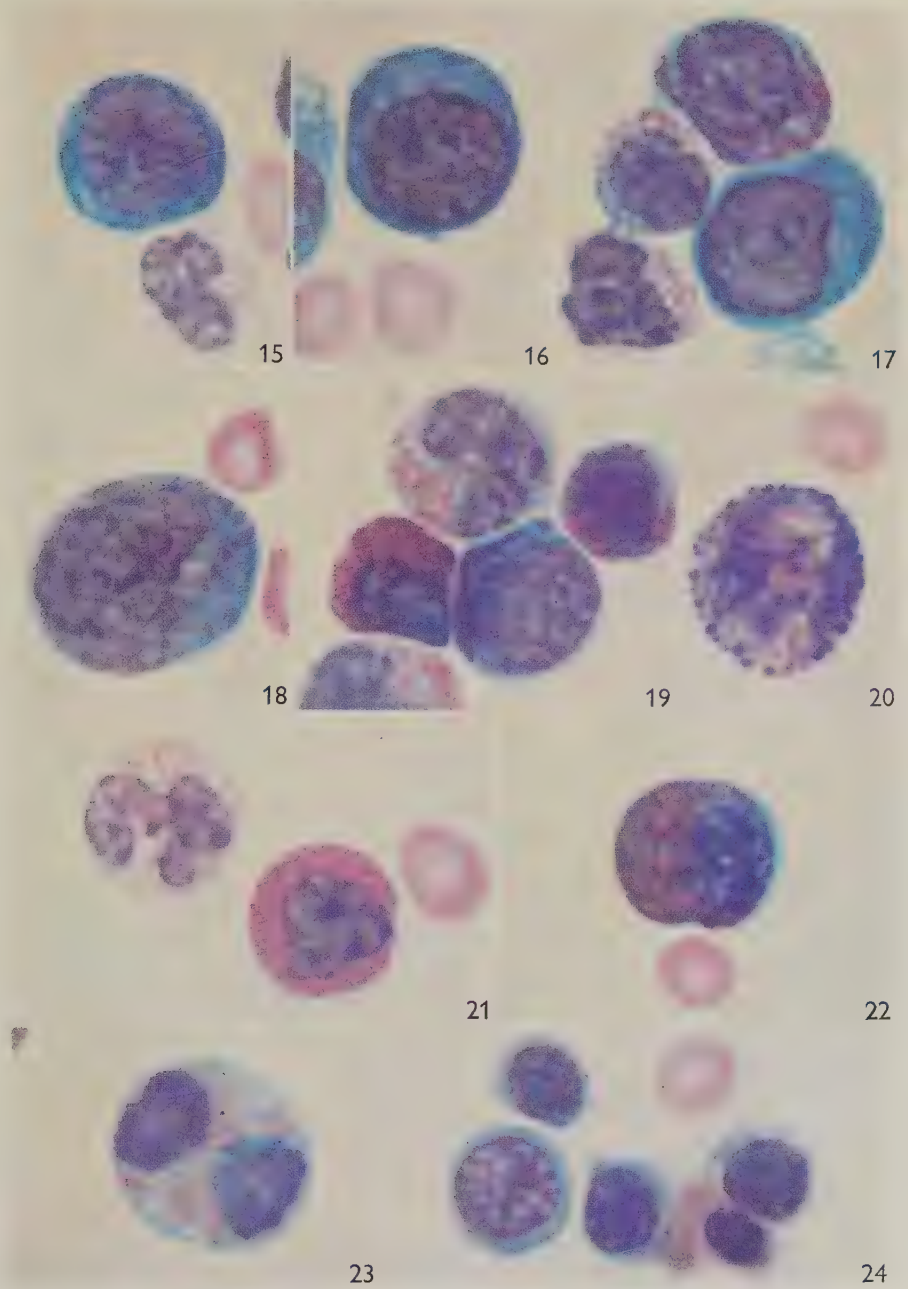
## PLATE 2

- Fig. 15. From normal guinea-pig bone marrow ?myeloblast ?proerythroblast. The nucleus suggests a proerythroblast but the cytoplasm showed precisely the same staining as other cells which were undoubtedly myeloblasts. This cell then could be a myeloblast in early prophase or later telophase.
- Fig. 16. From bone marrow of normal guinea-pig. Myeloblast with nucleus resembling that of the reticulum cell.
- Fig. 17. Normal guinea-pig bone marrow. Very early promyelocyte with only a few granules. Swollen and pale nucleoli. Sharp nuclear border. Also one pseudo-eosinophil myelocyte and two metamyelocytes. The granules in the myelocyte give the impression of being more eosinophilic than those in the metamyelocytes.
- Fig. 18. Normal guinea-pig bone marrow. Large early myelocyte.
- Fig. 19. Normal guinea-pig bone marrow. Basophil myelocyte, two eosinophil metamyelocytes and one pseudo-eosinophil metamyelocyte.
- Fig. 20. Normal guinea-pig bone marrow. Basophil metamyelocyte.
- Fig. 21. Normal marrow. Mature pseudo-eosinophil and eosinophil granulocyte.
- Fig. 22. Normal marrow. Eosinophil myelocyte.
- Fig. 23. Pseudo-eosinophil myelocyte or metamyelocyte in mitosis. Normal marrow.
- Fig. 24. Normal marrow. Red cells in different stages of maturation.













# THE VENOUS DRAINAGE OF THE LEFT ATRIUM

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With the exception of the fairly constant vena obliqua atrii sinistri there are no large calibre veins draining the left atrium. It is generally assumed that this lack is made up by the venae minimae of Thebesius (1708). Some, however, have held that these tiny channels were merely blind diverticulae. Haller (1757) contended that the discharge of venous blood into the left heart was unlikely on physiological grounds. That these channels do indeed communicate with the general system was demonstrated, by injection methods, by Thebesius (1708) and Langer (1880). More recently, these findings have been confirmed by Gross (1921); whilst Grant (1926) has shown in the rabbit embryo that the venae minimae develop independently as outgrowths from the lining of the atrium, but subsequently fuse with the main coronary venous system.

The introduction of an efficient self-illuminating cardioscope by Butterworth (1951) has enabled the interior of the living heart to be inspected, and has given final proof that these channels function actively as veins. Observations were made first on dogs (Butterworth, 1951) and later by a surgeon (Sellers, 1952) on four patients undergoing operations for the relief of mitral stenosis.

In each of the ten dogs examined streams of dark blood varying in size up to half a millimetre in diameter were seen issuing from the dead white endocardium of the atrial wall. The dark colour of this blood stands out clearly in contrast to the bright red blood filling the atrial chamber. These streams had a steady flow which did not vary with the state of contraction of the atrial wall and could be clearly followed for distances up to 1 cm. before they finally frayed out and merged into the surrounding blood. They had no regular distribution and were scattered at intervals of from 1 to 3 cm., being more numerous on the anterior wall and lower part of the inter-atrial septum. In the human subject it was of course impossible to carry out such full examinations as in the dogs, but similar streams of dark blood were seen on each occasion. In a greyhound the cardioscope was passed right through the mitral ring and the left ventricular wall explored. No streams of dark blood were seen. Whether this was due to the absence of venae or merely to difficulty in seeing them in the swirling ventricular content was not certain.

Only the crudest estimate of the quantity of venous blood discharged from this source could be made, but experiments *in vitro* with coloured fluids passed through fine hypodermic needles suggested that similar appearances would be given by streams of up to 2 cu.cm./min. It would therefore appear that the venae minimae play an appreciable part in the venous drainage of the left atrium.

In all the cases described the cardioscope was introduced via the left auricle. Thus no observation could be made on the venous drainage of that part of the heart itself. In certain cases in the human the best method of entry for the cardioscope may prove to be through the left inferior pulmonary vein. Opportunities for

studying the inner wall of the left auricle in the living may therefore occur in the near future.

#### SUMMARY

1. The existence of Thebesian veins is discussed.
2. Final proof, by direct observation, of their active function is given.
3. Their part in the venous drainage of the left atrium is commented upon.
4. The difficulties of inspecting the inner wall of the left auricle in the living are discussed, and hopes expressed that new methods of introducing the cardioscope may allow them to be overcome.

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# AN EXPERIMENTAL STUDY OF THE FUNCTIONS OF THE LUMBRICAL MUSCLES IN THE HUMAN HAND

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## INTRODUCTION

The many conflicting views expressed in the literature regarding the functions of the mm. lumbricales have been reviewed by Sunderland (1945), prior to giving his own observations as to their function based on evidence of nerve injuries. More recently, Braithwaite, Channell, Moore & Whillis (1948) have presented an entirely new concept of lumbrical muscle function founded on both experimental and clinical evidence. In order to assess the validity of the different functions attributed to the lumbrical muscles, their activity has been studied here by (1) electromyography, and (2) electrical stimulation. Normal subjects, not subjected to any form of anaesthesia, were used in all the experiments, a point considered essential both to preserve the normal functional activity of all muscles associated with the mm. lumbricales in digital movements, and to avoid certain of the pitfalls encountered by previous workers in this field.

## ELECTROMYOGRAPHY

Muscle action potentials were obtained from concentric needle electrodes constructed from 40 s.w.g. enamelled copper wire (diameter  $120\ \mu$ ), cemented into a size 20 hypodermic needle. Photographic recordings were obtained using an a.c. coupled amplifier and a cathode-ray tube. Although all the lumbrical muscles were examined to exclude any individual variation, the second lumbrical muscle was chosen in most experiments for the following reasons:

(1) It is the easiest of the four muscles to contact with accuracy at a depth of 5–7 mm. The anatomical accuracy of position was initially tested in the cadaver, by inserting the needle and then checking the position of its point by dissection. So long as the position of the flexor tendons can be found by palmar observation or palpation, insertion of the needle into the muscle is quite a simple procedure.

(2) It is remote from both the thenar and the hypothenar muscle groups, so that electrical interference from these is rendered unlikely.

(3) It is separated from the mm. interossei by the transverse head of m. adductor pollicis. The superficial position of the needle in relation to this last muscle can be demonstrated by inserting it more deeply until electromyographic recordings are obtained during thumb adduction, using the same amplification as that required for lumbrical recordings. The needle is then withdrawn to the correct depth for the lumbrical muscle.

With the needle within the second lumbrical muscle, and using the necessary amplification for recording therefrom, no electrical spread was detectable from the transverse head of m. adductor pollicis or from the other thumb muscles when these

were acting with maximal force (Fig. 1).<sup>\*</sup> Therefore no electrical activity was expected to spread from the more deeply lying interosseous muscles. This expectation was confirmed by taking simultaneous recordings from the second lumbrical muscle and the second dorsal interosseous muscle, using identical electrodes for both. The degree of activity observed in various movements of the fingers showed markedly different results in the two muscles. For example, in full extension at the metacarpo

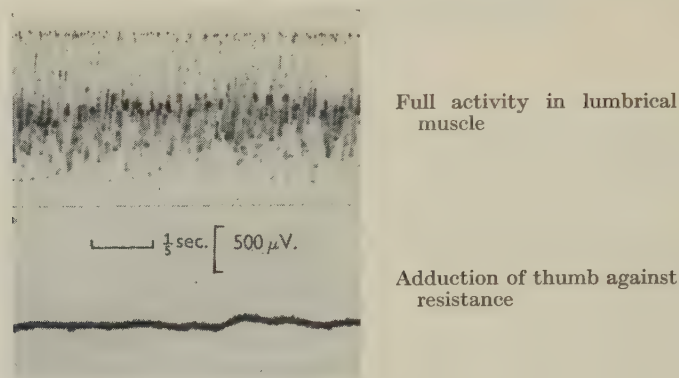


Fig. 1. Electrodes in second lumbrical muscle.

phalangeal and interphalangeal joints the lumbrical muscle showed a high level of electrical activity, whereas the interosseous muscle showed little or none. In full flexion at the same joints the lumbrical muscle showed no, but the interosseous muscle a marked, activity. It was found that in order to obtain the most satisfactory recordings the needle had to be placed within the lumbrical in as distal a position as possible. This procedure was rendered necessary by movements of the m. flexor digitorum profundus tendon which affect the position of the origin of the lumbrical muscle. There is, for instance, a tendency to bow-stringing on flexion which leads to variation in depth of the lumbrical in relation to the needle point if this be inserted too proximally; there is also considerable excursion of the needle point during flexion and extension, especially at the metacarpo-phalangeal joint.

#### OBSERVATIONS

With the hand in the normal position of rest no action potentials were recorded from the lumbrical muscle.

*Firm extension of the interphalangeal joints* produced a high level of activity in the lumbrical muscle, irrespective of the position of the metacarpo-phalangeal joint (Fig. 2). This high level was maintained even in hyperextension at the metacarpo-phalangeal joint. One subject examined was able to hyperextend this joint actively to 45° with very little change in lumbrical activity throughout the whole range of movement (approximately 130°) at the metacarpo-phalangeal joint.

\* The electrical recording during full activity in the lumbrical muscle in this figure, and also the third recording in Fig. 2, show overloading of the amplifier. This overloading was deliberate in order to show possible evidence of low levels of electrical activity in other movements.

*Relaxation of interphalangeal extension*, whatever the position of the metacarpo-phalangeal joint, produced an immediate reduction in the electrical activity in the lumbrical muscle. So marked was this that, even though no true digital flexion accompanied relaxation of extensor tension, the recorded action potentials were reduced almost to nil. Hence it was extremely difficult to assess variation of lumbrical action potentials in relation to the position of the metacarpo-phalangeal joint. Although subjects endeavoured to maintain a steady interphalangeal extensor tension throughout the whole range of movement at the metacarpo-phalangeal joint, results were rather variable, not only from subject to subject but also periodically in the same subject.

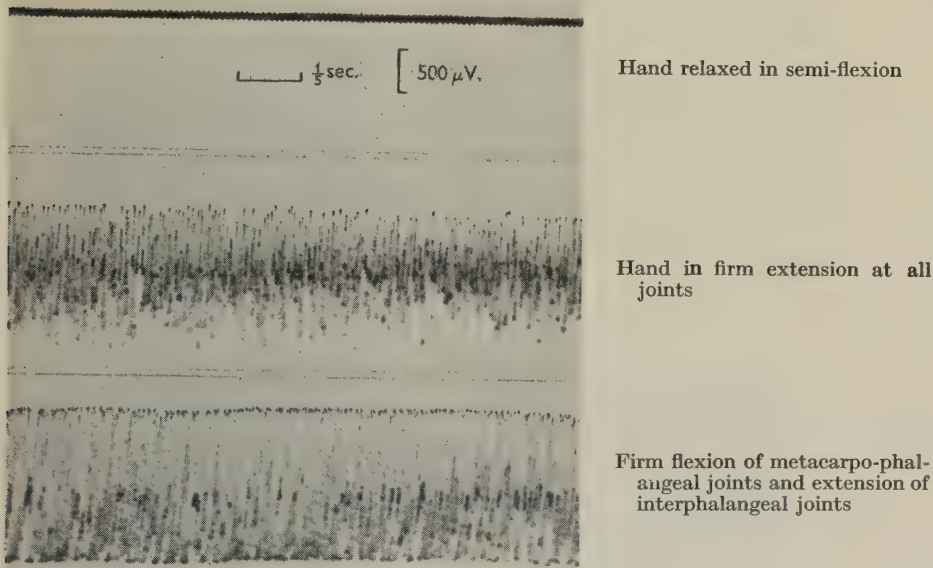


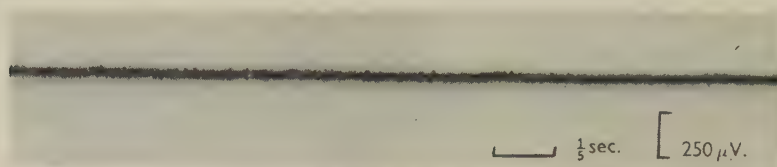
Fig. 2. Electrodes in second lumbrical muscle.

*Flexion at the metacarpo-phalangeal joint with interphalangeal extension* did appear to give a slightly higher level of lumbrical electrical activity than in metacarpo-phalangeal extension, but this observation must be treated with extreme caution in view of the difficulties stated above, and also in view of possible variations due to bow-stringing of the m. flexor profundus tendon. If flexion at the metacarpo-phalangeal joint is carried out with the interphalangeal joints extended, against a resistance applied at the finger tips, the degree of lumbrical activity is found to be reduced below the above level. Furthermore, activity in the lumbrical muscle during this movement could be completely abolished in subjects who relaxed their interphalangeal extension and allowed the digital position to be maintained by the flexion at the metacarpo-phalangeal joints and the counter-pressure at the finger tips.

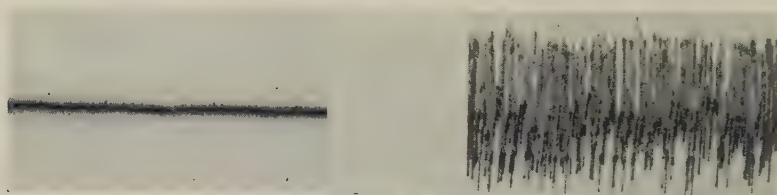
*Metacarpo-phalangeal flexion with interphalangeal flexion* at no time produced any degree of electrical activity in the lumbrical muscle. This remained true even when such movement was made against maximal resistance.



*Opposition of the medius digit to the thumb* likewise produced no evidence of electrical activity in the lumbrical muscle, no matter what the intrinsic position of the thumb, so long as the interphalangeal joints of the medius were not actively



Forced opposition against thumb in extension  
compared with



Muscle relaxed

Muscle fully active

Fig. 3. Second lumbrical muscle.



Fig. 4. The position of forced opposition against the thumb in extension  
in which the tracing in Fig. 3 was taken.

extended in the movement (Figs. 3, 4). If, however, active interphalangeal extension did occur in the finger during this movement, then electrical activity appeared in the muscle.

Radial deviation of the digit produced no electrical activity of the lumbrical muscle in any position of the metacarpo-phalangeal joint so long as no such activity was present before the initiation of this movement. (Radial deviation here includes that peculiar excursion of the proximal phalanx upon the curved metacarpal head which has been designated 'digital rotation' (Braithwaite *et al.* 1948).) Radial deviation carried out against resistance with the interphalangeal joints passively extended produced relatively little variation in the degree of lumbrical electrical response. In some subjects indeed a reduction of response was noted (Fig. 5).

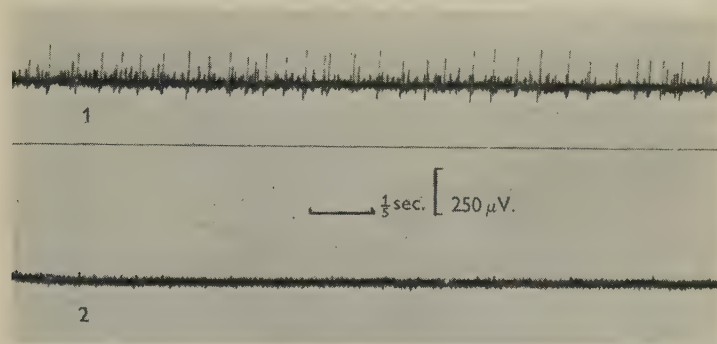


Fig. 5. Second lumbrical muscle: 1, relaxed in extension with ulnar deviation; 2, radial deviation against resistance.

The position of the wrist joint in no way affected the electrical activity of the lumbrical muscle engendered by interphalangeal joint extension.

#### STIMULATION

The lumbrical muscle was stimulated using for one electrode the core wire of the concentric needle electrode employed for the electromyographical study. This gave an effective stimulating area of the end of this wire of diameter  $120\mu$  bevelled in one direction at approximately  $45^\circ$ . A large plate on the forearm was used for the other electrode, and was moved from place to place to exclude possible variation in stimulation effect. As would be expected from the small size of the intramuscular electrode, a high concentration of current was produced at this point: this concentration remained constant whatever the position of the plate-electrode. The voltages employed were those found to be sufficient for effecting lumbrical contraction yet low enough to obviate spread to neighbouring muscles. The stimulator employed gave a condenser shock discharge of  $\frac{1}{2}$  msec. duration, at frequencies of between 50–100 per second, at an amplitude up to 60 V. d.c., from an internal impedance of about  $600\Omega$ . The wave form was that shown in Fig. 6. Owing to the electronic circuit involved this wave did not show the classical sharp rise and exponential fall, though it did not differ greatly therefrom. The back edge of the pulse was more linear than exponential, due to inductance loading in the stimulator output circuit. This was also responsible for the small negative deflexion which

followed the main pulse. The effective stimulation time was approximately  $\frac{1}{3}$  msec. and the negative deflexion was shown to be too small to produce a stimulation.

Two stimulation currents were used, viz. (a) the maximum output of the stimulator, and (b) a lower current of about 30% of maximum output which was the minimum output capable of producing consistent stimulation of the muscle.

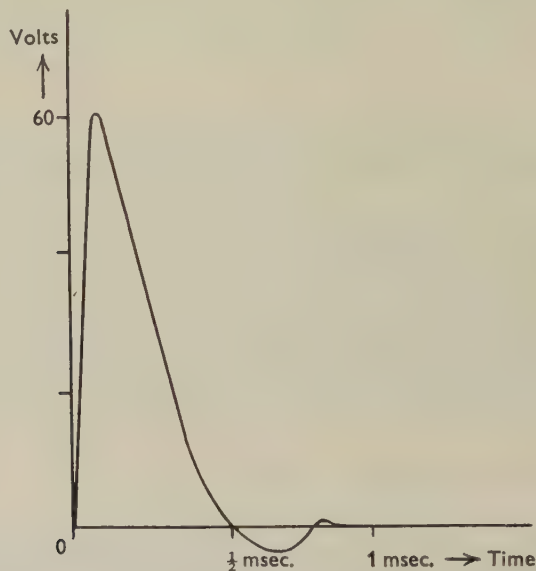


Fig. 6. Electrical wave-form given by the stimulator employed.

*A high level of stimulation* applied to the relaxed muscle produced firm extension at the interphalangeal joints, with flexion at the metacarpo-phalangeal joint to approximately  $80^\circ$  (Fig. 7). It was visually obvious that the initial lumbrical activity was always one of extension at the interphalangeal joints (no matter what the position of the metacarpo-phalangeal joint) followed by secondary flexion of the metacarpo-phalangeal joint.

*A low stimulatory current*, however, produced results which demonstrated the components of lumbrical function more simply. Such a current produced interphalangeal extension, the metacarpo-phalangeal joint retaining mainly its initial position. Full flexion at this latter joint occurred only if a higher current were passed. A certain small degree of metacarpo-phalangeal flexion invariably accompanied interphalangeal extension—relatively little if the metacarpo-phalangeal joint were initially extended, but rather more if it were partially flexed. The movement was in effect identical with the voluntary movement of interphalangeal extension in which it is extremely difficult to obviate a slight metacarpo-phalangeal flexor component. This flexor movement was, however, an essential part of the main movement of digital extension and differed from the pure flexion occurring after interphalangeal extension with higher currents.

No evidence of radial deviation was discernible.



Active control of the fingers against lumbrical stimulation was attempted by the subject in every case. It was found extremely difficult, or even impossible, to prevent extension of the interphalangeal joints if an adequate lumbrical stimulation current



Fig. 7. Stimulation of the second lumbrical muscle: 1, hand relaxed; 2, muscle stimulated.

were used. When the interphalangeal joints were extended it was impossible to flex them against such stimulation. However, the effects of lumbrical stimulation upon the metacarpo-phalangeal joint differed. Stimulation led to metacarpo-

phalangeal joint flexion if the interphalangeal joints were extended and the voltage was high enough, yet it was not difficult voluntarily to resist this movement. In fact the metacarpo-phalangeal joint could execute its full range of movement even when considerably higher currents were used than those necessary to render voluntary control of the interphalangeal joints impossible. Freedom of movement in a radial or ulnar direction, or of 'rotation', did not appear to be affected.

*Sensations produced during lumbrical stimulation* were experienced by the subjects and were found to be of a purely flexor-extensor character. Sensations of digital deviation or 'rotation' were entirely absent. On the other hand, stimulation of an interosseous muscle produced the remarkable sensation of complete loss of control due to deviation in the digit, a sensation absent in pure flexion-extension movements and entirely absent on lumbrical stimulation.

#### DISCUSSION

*Interphalangeal extension.* The evidence obtained from electromyographical investigation indicates that the lumbrical muscle acts primarily to produce extension of the interphalangeal joints, and this view is supported by the fact that similar activity is produced in the muscle on electrical stimulation. But it is apparent, both by observation and by palpation, that m. extensor digitorum communis is also contracting during active interphalangeal extension. Furthermore, Braithwaite *et al.* (1948) have shown that digital extension is an inefficient (but possible) movement in experimental paralysis of the long extensor. Therefore, the lumbrical muscle can be said to carry out this movement efficiently only in association with a normally acting m. extensor digitorum communis. Sunderland (1945) has suggested that an important aspect of lumbrical-interosseous extension at the interphalangeal joints is the prevention of hyperextension of the proximal phalanx by the m. extensor digitorum communis, and that this preventive action allows a more efficient pull to be transmitted to the dorsal expansion which then operates directly upon the interphalangeal joints. This view is herein supported in so far as the lumbrical muscles are concerned, on the evidence of the primary metacarpo-phalangeal flexor component occurring during digital extension after stimulation by even weak currents.

The prevention of hyperextension in digital extension cannot be the sole function of the lumbrical muscle, for such hypothesis fails to explain the relatively powerful extensor effect resulting from direct lumbrical stimulation, compared with the weaker secondary metacarpo-phalangeal flexor effect which follows the completion of extension. Furthermore, if the extensor effect were due solely to this preventive action, it would be reasonable to expect evidence of lumbrical muscle action in metacarpo-phalangeal flexion irrespective of the position of the interphalangeal joints, and such evidence has not been found in the present studies.

It appears, therefore, that the lumbrical muscle is an active extensor of the interphalangeal joints, and that its action is assisted very considerably by the m. extensor digitorum communis but only when the latter's hyperextensor effect upon the metacarpo-phalangeal joint is neutralized by the lumbrical or interosseous muscles.

*Metacarpo-phalangeal flexion* appears to be carried out by the lumbrical muscle only when the interphalangeal joints are extended. This view is strongly supported

by the effect of lumbrical stimulation which results in flexion of the joint as a direct movement only subsequent to extension of the interphalangeal joints. The evidence of electromyography, though less convincing, does indicate the same conclusion, viz. that, although metacarpo-phalangeal flexion produces action potentials in the lumbricals only in active interphalangeal extension, the level of electrical activity appears to be higher in flexion than in extension of the metacarpo-phalangeal joint (Fig. 2). Although the value of this latter observation is not great it provides a certain supporting evidence.

*Accessory movements.* Present studies do not support the conclusions of Braithwaite *et al.* (1948) that the lumbrical muscles are active during digital opposition to the thumb or in digital radial deviation. The value of the lumbrical muscle as a radial deviator in the absence of interosseous muscle action cannot be assessed from these experiments.

#### SUMMARY

The actions of the lumbrical muscle have been studied experimentally by electromyography and by electrical stimulation.

The principal action of the muscle is that of an extensor of the interphalangeal joints, assuming that both the long muscles in union with which it operates maintain normal function. It is a weak flexor of the metacarpo-phalangeal joint, but effectively so only in interphalangeal extension. It appears to have no effect on 'rotation' or radial deviation of the finger and is not used in opposition of the finger to the thumb except in full and active extension of the digital interphalangeal joints.

We wish to express our thanks to Prof. A. A. Harper for allowing us facilities to carry out this work in the Department of Physiology; to Prof. J. Short and Dr T. E. Barlow for considerable assistance; and to Prof. A. J. E. Cave and Prof. J. Whillis for much helpful criticism and advice. Finally we thank the students who acted as subjects for this work, often with some marked discomfort.

Photographs are published by kind permission of Mr C. J. Duncan of the Photographic Department, King's College, University of Durham.

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## OBSERVATIONS ON THE CHROMAFFIN REACTION

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## INTRODUCTION

Since the work of Gerard, Cordier & Lison (1930), it has been generally accepted that the chromaffin reaction is an oxidative phenomenon and independent of the presence of chromium in the fixing solution. It has been suggested that an identical histological picture can be produced by fixing the adrenal medulla in either formol-potassium iodate or in formol-dichromate. Whilst investigating the disposition of the extra-adrenal chromaffin tissues in man and lower animals, the writer has been impressed with the marked difference in the colour (quantitatively and qualitatively) of the formol-iodate- or formol-dichromate-fixed adrenal medulla. As a result of these findings further investigations have been carried out, the primary object being to ascertain whether or not chromium is fixed by the formol-dichromate-treated adrenal medulla.

So far back as 1865, Henle noted a brown coloration of the chromic acid- or chromate-fixed adrenal medulla. A similar change was observed by Stilling (1890) in certain extra-adrenal cells which were closely associated with the prevertebral sympathetic plexuses and which are now usually referred to as sympathetic paraganglia or para-aortic bodies; he referred to the colour change as the 'chromaphil' reaction.

Kohn (1900, 1902, 1903) published extensive reviews of the sympathetic nervous system and associated structures, incorporating many personal observations on the development of the adrenal medulla and 'paraganglia'. In these works he referred to the brown coloration of cells after chrome fixation as the 'chromaffin' reaction—a term which has remained in general use up to the present time, in spite of the various alternative names which have been put forward.

The association between a positive chromaffin reaction and the presence of a pressor substance in extracts of the affected tissues has been recognized for many years. Oliver & Schäfer (1894) reported the presence of a pressor substance in extracts of the adrenal medulla, and a similar pressor substance was extracted from extra-adrenal chromaffin tissue by Biedl & Wiesel (1902).

Ogata & Ogata (1923) reviewed the literature relating to the chromaffin reaction, and compared the changes which occur in the adrenal medulla after fixation in formol-dichromate with those produced by mixing solutions of potassium dichromate and adrenaline. A brown precipitate was observed *in vitro*, which by analysis was shown to be an oxide of chromium. It was concluded that this precipitate was responsible for the colour change in the cells of the adrenal medulla.

Gerard, Cordier & Lison (1930) carried out further investigations into the nature of the chromaffin reaction. These workers reported that a positive chromaffin reaction could be produced using either formol-potassium dichromate or formol-potassium iodate as a fixative. They performed a series of experiments *in vitro*, and

reported that the reaction obtained by mixing a solution of adrenaline with one of potassium dichromate could be divided into two stages:

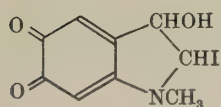
(a) Within 15 min. of mixing the two solutions a preliminary oxidation of adrenaline occurs with the formation of a reddish brown soluble compound. The colour can be removed by treatment with sodium hyposulphite, ammonia or bromine water.

(b) After some hours a brown precipitate develops which cannot be removed by bromine water, sodium hyposulphite or ammonia.

These changes were compared with those produced by mixing minced adrenal medulla with potassium dichromate. Gerard *et al.* found that the medullary tissues became brown after 15 min. and that decolorization could be brought about by adding bromine water. Sodium hyposulphite and ammonia were without effect.

As a result of these findings, Gerard *et al.* (1930) concluded that the reaction between potassium dichromate and the adrenal medulla coincided with the initial oxidation stage of adrenaline and potassium dichromate, and that a similar reaction could be produced by fixing medullary tissue in formol-potassium iodate. The reaction was thought to be independent of the presence of chromium and it was suggested that the term 'chromaffin' should be replaced by 'pleochrome'. The latter work was repeated by Bennett (1941) with the same conclusions and he suggested that the term 'chromaffin reaction' should be replaced by 'fuscogenic reaction'.

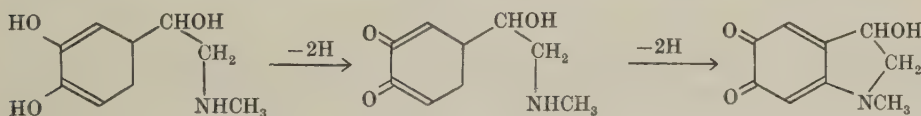
During recent years the oxidation products of adrenaline have been investigated by a number of workers. Richter & Blaschko (1937) showed



I

that the product of adrenaline and potassium iodate is an iodine-containing oxidation product of adrenaline with a formula I.

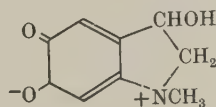
Green & Richter (1937) suggested that the oxidation of adrenaline (II) usually resulted in the formation of an ortho-quinone (III) and subsequently a dihydroindole derivative, adrenochrome (IV). This was modified by Harley-Mason (1948) who suggested that adrenochrome existed primarily as the zwitterion *p*-quinoneimine (V).



II

III

IV



V

## MATERIALS AND METHODS

(1) Solutions containing different concentrations of adrenaline and noradrenaline have been treated with 3% potassium chromate, dichromate and iodate.

(2) Rabbit adrenal glands were fixed in mixtures of 5 % formaldehyde with the addition of 3 % potassium dichromate or 3 % potassium chromate or 3 % potassium iodate. Paraffin sections were made and examined unstained and after staining with Ehrlich's haematoxylin or Giemsa. The Giemsa-stained sections (after 24 hr. staining) were differentiated in

(i) 400 ml. distilled water to which 2 ml. glacial acetic acid had been added. Approximate time,  $1\frac{1}{2}$  min.

(ii) 90 % alcohol. Approximate time, 1 min.

(iii) Absolute alcohol, two changes. To the first absolute 1 ml. of glacial acetic acid per 400 ml. was added. Approximate time, 1 min. in each. The differentiation was controlled microscopically. Sections were mounted in neutral balsam.

Some unstained sections were treated with normal hydrochloric acid, ammonia and bromine water.

(3) Formol-dichromate-fixed adrenal glands were washed for 24 hr. and subsequently dehydrated in alcohol. The cortex was dissected free from the medulla, and known amounts of each tested for chromium, (a) by fusing with borax, (b) by fusing with sodium peroxide which converts any chromium into the chromate. This was dissolved in water and acidified with hydrochloric acid. Three drops of diphenylcarbazide (made by dissolving 0.2 g. crystalline diphenylcarbazide in 10 ml. acetic acid and diluting with 100 ml. water) were added to 10 ml. of the acidified solution and the intensity of the resulting violet-red colour estimated colorimetrically.

(4) Radioactive chromium (Cr 51) was used to prepare a solution of potassium dichromate. Cr 51 has a half-life of 26.5 days and decays by K capture and the internal conversion of  $\gamma$  photons, with the release of X-rays.

20 ml. of 2.0 % potassium dichromate were prepared which had an activity of 1  $\mu$ curie/ml. Formaldehyde was added and rabbit adrenal glands fixed for 18 hr. by immersion.

The tissue was washed for 24 hr., dehydrated and paraffin sections prepared. These were cut at 8–10  $\mu$ . The sections were mounted on glass slides which had been treated with an aqueous solution of 1 % gelatine and 0.1 % chrome alum (Doniach & Pelc, 1950). The slides were allowed to dry, after which Kodak autoradiograph stripping emulsion was floated on to the sections in the dark-room, using a safety light. The slides were dried and stored in the dark at 34° F. for 3 months, single slides being developed from time to time to assess the effect on the photographic emulsion. The slides were developed in Kodak D 61a and fixed in acid hypo. Some were examined by phase contrast with emulsion and sections superimposed; others were examined after the emulsion had been stripped from the underlying sections (Text-figs. 2, 3). Control sections fixed in non-radioactive formol-dichromate were treated in the same way so as to exclude the possibility that the silver reduction, if any, was due to the quinone-like substances present in the adrenal medulla.

(1) (a) If 1 ml. of adrenaline or noradrenaline (1 mg./ml.) and 1 ml. of 2 % potassium dichromate are mixed a reddish brown solution results. The colour begins to develop within 1 min. and is maximal after 15 min. After about 45–60 min. a dark brown amorphous precipitate is formed. This contains chromium and is probably an oxide of chromium.



The initial colour can be abolished by adding bromine water; the precipitate is unaffected by this reagent.

(b) The addition of 1 ml. of adrenaline or noradrenaline (10 mg./ml.) to 1 ml. of 2% potassium dichromate results in a reddish brown coloration, and a dark brown precipitate appears within 1 min.; these are maximal within 15 min.

It is, therefore, apparent that the formation of the precipitate depends upon the concentration of adrenaline. The precipitate is probably an oxide of chromium produced by the reduction of dichromate.

(c) When adrenaline or noradrenaline (1 mg./ml. or 10 mg./ml.) is added to potassium chromate the changes produced are similar to those reported in 1 (a) and (b).

(d) If adrenaline or noradrenaline is added to 3% potassium iodate a bluish red solution is formed; this is quite different in colour from that produced by chrome compounds. This coloration is followed after a variable time (depending upon the concentration of the reagents) by the deposition of bluish red needle-shaped crystals of iodo-adrenochrome.

2 (a) Formol-dichromate or formol-chromate fixation produces a yellow-brown coloration of the adrenal medulla which is apparent after 5 min. and maximal after 15–30 min. The colour is not water soluble, it is removed by immersing in bromine water, but not by placing in ammonia or normal hydrochloric acid. The pH of the solution (in the range 3–8) has little effect upon the intensity of the reaction, and a maximal reaction can be obtained with unbuffered solutions of formol-dichromate. The type of formaldehyde used, i.e. neutralized or ordinary, has no obvious effect upon the intensity of the reaction. Formol-dichromate prepared with the neutralized reagent is, however, preferred as it is more stable.

In unstained sections and those stained with haematoxylin the typical yellow-brown chromaffin reaction is observed in the cells of the medulla (Pl. 1, fig. 1). Red blood corpuscles are similarly coloured. When Giemsa is used the cytoplasm of the medullary cells is stained a blue-green colour, whilst the red blood corpuscles become bright red (Pl. 1, fig. 3).

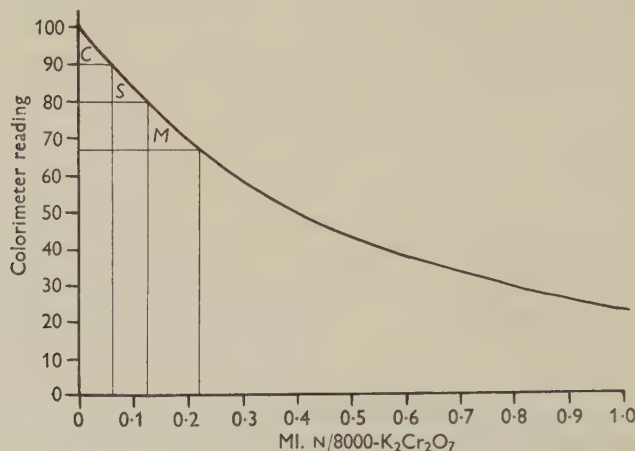
(b) Formol-iodate fixation gives rise to a brown coloration of the adrenal medulla which is visible after 5 min. and maximal after 30 min. The colour is different qualitatively and quantitatively from that produced by chrome fixation; it is most intense if the pH is within the range 5–6.5 but is never as intense as that produced by chrome-fixed material and is without the yellowish tint of the latter.

Giemsa stains the cytoplasm of medullary cells a bluish colour, the appearance being somewhat similar to that observed after fixing in formol-dichromate.

3 (a) *Fusion of cortex and medulla with borax.* If equal amounts of cortex and medulla of a dichromate-fixed adrenal gland are fused with borax, an intensely green bead is produced by the medulla whilst that produced by cortex is colourless or only faintly coloured.

(b) *Assessing the presence of chromium by diphenylcarbazide.* The intensity of the colours produced by known weights of adrenal cortex and medulla were compared, and a comparison made between these and known solutions of potassium dichromate (Text-fig. 1). It was considered that medullary tissue may give a relatively high reading because of its greater blood content, and in order to assess this effect pieces

of spleen were fixed in formol-dichromate and treated in the above way. The results (Table 1) indicate that adrenal medullary tissue contains four times as much chromium as does an equal amount of adrenal cortex and twice that of the spleen. The chromium content of the cortex and spleen is due mainly to incomplete washing.



Text-fig. 1. Extracts of adrenal cortex, adrenal medulla and spleen were treated with diphenyl-carbazide. The intensity of the colour produced in each case was estimated by comparison with similarly treated known solutions of potassium dichromate. Ordinate, colorimeter reading; abscissa = ml. of  $N/8000-K_2Cr_2O_7$ .

TABLE 1

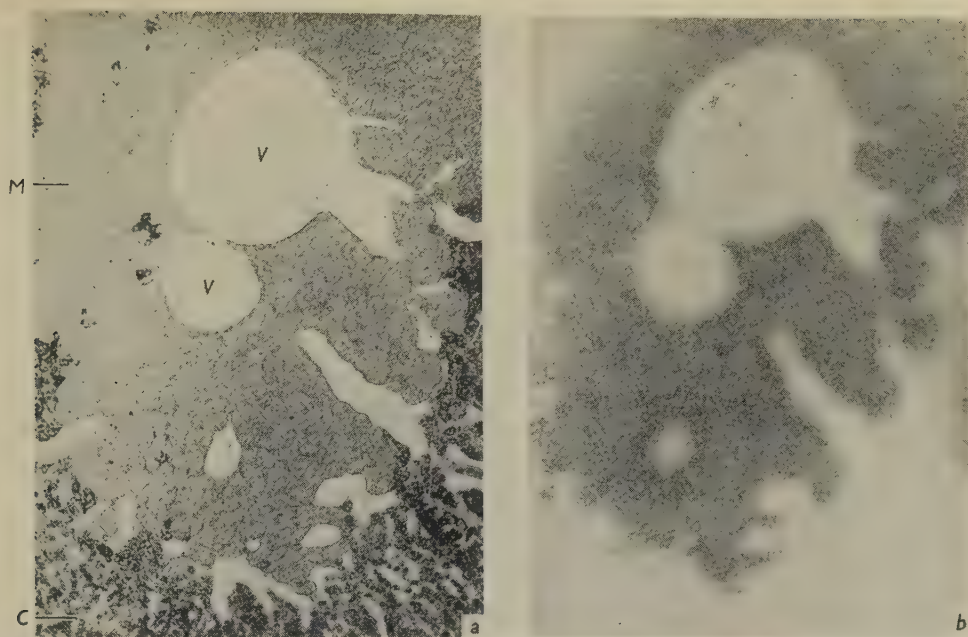
	Tissue	Weight (mg.)	Equivalent	Equivalent
			$N/8000-K_2Cr_2O_7$ (ml.)	$N/8000-K_2Cr_2O_7$ (ml./10 mg. tissue)
Expt. I	Cortex	2.7	0.31	1.1
	Medulla	2.7	1.03	3.4
	Spleen	2.8	0.63	2.2
Expt. II	Cortex	14	1.60	1.1
	Medulla	7	3.20	4.3
Expt. III	Cortex	2.5	0.25	1.0
	Medulla	2.5	0.97	3.9
	Spleen	2.5	0.44	1.7

(4) Autoradiographs were prepared after fixing in radioactive formol-dichromate. Definite reduction of the emulsion occurred over the cells of adrenal medulla (Text-figs. 2, 3), but no significant reduction occurred elsewhere on the sections. The intensity of the reduction could be correlated with the duration of exposure. All control sections either failed to give reduction or produced a very faint fogging which was of equal intensity in both cortex and medulla.

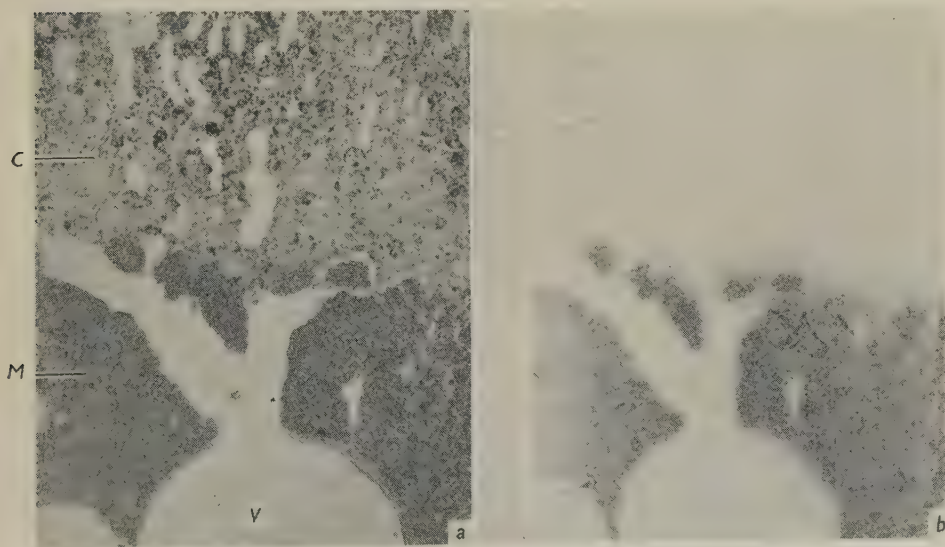
These findings indicate that Cr 51 is fixed by cells of the adrenal medulla.

#### DISCUSSION

The presence of chromium in the dichromate-fixed adrenal medulla is indicated by both chemical analysis and the use of radioactive potassium dichromate. The colour produced is removed by bromine water, whereas the oxide of chromium which is



Text-fig. 2. *a*, unstained section of rabbit adrenal gland fixed in radioactive formol-dichromate; *M*, medulla; *C*, cortex; *V*, venous sinuses.  $\times 64$ . *b*, autoradiograph of the previous section. Blackening has occurred in the portion of the film which was in contact with medullary cells.



Text-fig. 3. *a*, unstained section of rabbit adrenal gland fixed in radioactive formol-dichromate. *M*, medulla; *C*, cortex; *V*, venous sinuses.  $\times 88$ . *b*, contact autoradiograph of the previous section. Blackening has occurred in the portion of the film which was in contact with the adrenal medulla.



deposited *in vitro* is unaffected; this finding is in agreement with the work of Gerard *et al.* (1930), and indicates that the oxide is not responsible for the histological reaction.

The 'true' chromaffin reaction, viz. the intense yellow-brown coloration of the cells of the adrenal medulla can only be produced by the use of fixatives which incorporate chromium-containing oxidizing agents. It can be produced by both formol-potassium dichromate and formol-potassium chromate, the former being the better fixative. As observed by Pearse (1953), the reaction produced by fixation in formol-iodate is maximal in buffered solutions (pH 5-6.5); this is, however, less intense and without the yellow tinge of the chrome-fixed material. Formol-iodate is, therefore, of less value in routine histological work.

The compound produced by fixation in formol-dichromate is likely to be an oxidation product of adrenaline and noradrenaline to which chromium is linked, i.e. a chrome adrenochrome; unlike adrenochrome it is not affected by treatment with ammonia or hydrochloric acid. A further evaluation of the chemical nature of this compound is outside the scope of this article.

The intensity of the chromaffin reaction in the normal adrenal gland depends in large measure on the method of fixation; it is much more pronounced in the perfused glands (Pl. 1, fig. 1) than in whole glands fixed by immersion. When fixation is by immersion much of the colour is found in the venous sinuses and little in the actual medullary cells (Pl. 1, fig. 2). This is probably due to the passage of pressor amine from the cells to sinuses prior to the penetration of the fixative. Post-chroming has been found to be of little or no value. The chrome adrenochrome is itself soluble *in vitro* and it would, therefore, seem likely that it would be rapidly lost by washing; the fact that this does not happen indicates that it is fixed in some way in the cells and in the blood plasma, possibly by some union with cell or plasma protein.

After formol-dichromate fixation the cytoplasm of the adrenal medullary cells shows a fine diffuse granularity with a superimposed yellowish brown coloration of both cytoplasm and nucleus. In the venous sinuses of inadequately perfused or non-perfused glands a non-granular 'chrome lake' exists.

The chromaffin reaction is commonly used as a histochemical test for adrenaline or noradrenaline. It cannot be regarded as specific because red blood corpuscles and granules of the entodermal argentaffin cells are also coloured brown by formol-dichromate fixation. Lipochrome pigments do not cause difficulties in paraffin sections. The argentaffin cells can be readily recognized by their position and if necessary by the use of various stains (Pearse, 1953); one has, therefore, to distinguish between the yellow-brown colour of the red blood corpuscles and of phaeochromocytes. In general the differentiation between red blood corpuscle and phaeochromocyte is not difficult, but if the blood corpuscles are superimposed upon other tissues a false positive chromaffin reaction may be diagnosed. This difficulty can be easily overcome by staining with Giemsa and differentiating in an acid medium, when the red blood corpuscles become bright red and the phaeochromocytes (intra- and extra-adrenal) become greenish blue (Pl. 1, fig. 3).

The coloration of the medullary cells may be accentuated and/or modified by treating the sections with 1% sulphanilic acid (Bayer, 1909), silver impregnation (Kutschera-Aichbergen, 1922), Schmore's ferric chloride-ferricyanide reagent,

Goldner's modification of Masson's trichrome stain (Bennett, 1941) or methylene blue (the agent responsible for the greenish blue colour produced by Giemsa). Lillie (1948) reported that chromaffin granules are P.A.S. positive. Using adrenal glands of man, cat, guinea-pig and rabbit, the writer found that the application of the P.A.S. technique to formol-dichromate- and formol-iodate-fixed sections of  $4\mu$  thickness resulted in a faint red-violet stain of the cytoplasm of the medullary cells; the chromaffin granules were not affected; this finding is in accord with the observations of Pearse (1953). When sections of over  $6\mu$  were used the background stain of the cytoplasm imparted a reddish brown hue to the chromaffin granules, which may be mis-diagnosed as a positive P.A.S. reaction. Hale's (1953) modification of the P.A.S. reaction, which consists of pretreatment with sodium hydroxide, also gives negative results.

Apart from the adrenochrome reaction described above, adrenaline-containing cells can also be visualized by the Vulpian reaction (1856) and Cramer's osmic acid method (1918). To produce the Vulpian reaction fresh frozen sections are treated with ferric chloride solution; a diffuse green colour results. The reaction is non-specific as it is given by other phenolic compounds such as the argentaffin granules (Cordier & Lison, 1930), and is of no localizing value as diffusion occurs.

Cramer's method consists of suspending thin slices of tissue in osmic acid vapour at  $37^{\circ}\text{C}$ . The osmic acid is reduced by the adrenaline-like compounds, and black granules appear in the medullary cells. The sections are subsequently embedded in paraffin and sectioned. Sections are treated with turpentine, which removes the lipid droplets whilst the adrenaline granules remain. This method is a useful accessory method for investigating the functional changes in the medullary cells in response to stimulation, etc., but is of little value for an adequate study of the distribution of chromaffin cells in large blocks of tissue. The reaction is again non-specific. Bennett (1941) stated that the osmic acid method was inferior to the chromaffin reaction in following the secretory cycle of adrenal medullary cells, but that it provided a useful accessory check. As all the methods in use are to some extent non-specific, the presence of adrenaline-secreting cells should be confirmed by using a combined histochemical and pharmacological approach, viz., chromaffin reaction plus assay of tissue for pressor amine. Where large numbers of sections are to be examined and knowledge of the general disposition of phaeochromocytes is required, the quickest and most satisfactory method is to fix in formol-dichromate, make serial paraffin sections and stain alternate slides with Giemsa and haematoxylin; eosin is best avoided as it masks the reaction. Neutral formaldehyde is used in preparing the fixative as it produces a more stable solution than the non-neutralized reagent.

During the present investigation and previous work on the para-aortic bodies (1952, 1953), a pressor substance (containing either adrenaline or noradrenaline) has been extracted from all tissues which give a positive chromaffin reaction except red blood corpuscles and argentaffin cells. The chromaffin reaction is unrelated to the specific pressor amine present; it is given by cells which yield a pressor amine which is almost all adrenaline (adult rabbit adrenal medulla) and by those which are entirely noradrenaline (human foetal and newborn rabbit para-aortic bodies).

Various alternative names have been suggested for describing the 'chromaffin

reaction'. Thus Gerard *et al.* (1930) suggested 'pleochrome', Van Campenhout (1946) the 'reaction of Henle', Bennett (1941) 'fuscogenic', these being prompted by the belief that the reaction was independent of the presence of chromium. The fact that chromium is fixed in the cells makes these alternatives unnecessary, and it is suggested that the well-established term 'chromaffin reaction' should be retained for referring to the yellow-brown coloration of cells which on extraction yield a pressor amine.

#### SUMMARY

Solutions of adrenaline or noradrenaline have been treated with formol-iodate or formol-dichromate and the colour reactions compared. A comparison has been made between formol-iodate- and formol-dichromate-fixed adrenal glands. The results of these investigations suggested that the compound produced in the adrenal medulla by dichromate fixation is different from that produced by iodate fixation.

Formol-dichromate-fixed adrenal glands have been chemically analysed for chromium content; some adrenal glands were fixed in radioactive formol-dichromate and autoradiographs prepared. The results of analysis and autoradiography indicate that after formol-dichromate fixation chromium is fixed by the cells of the adrenal medulla.

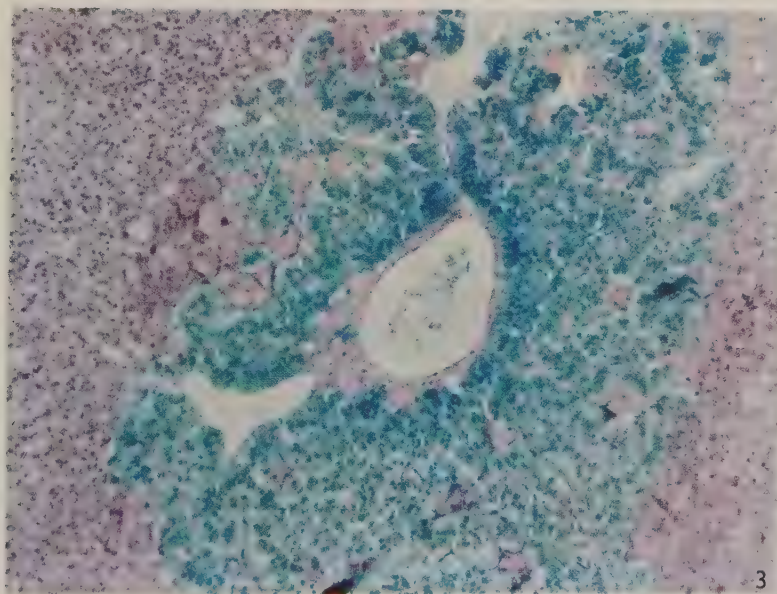
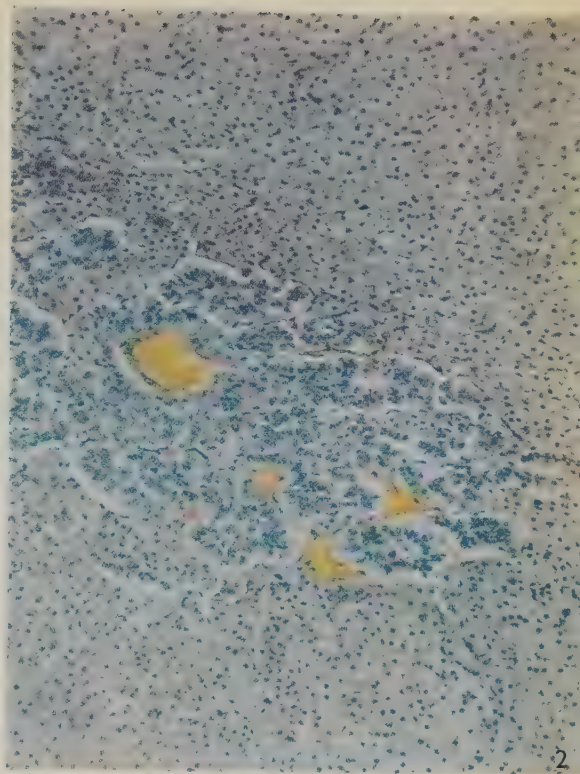
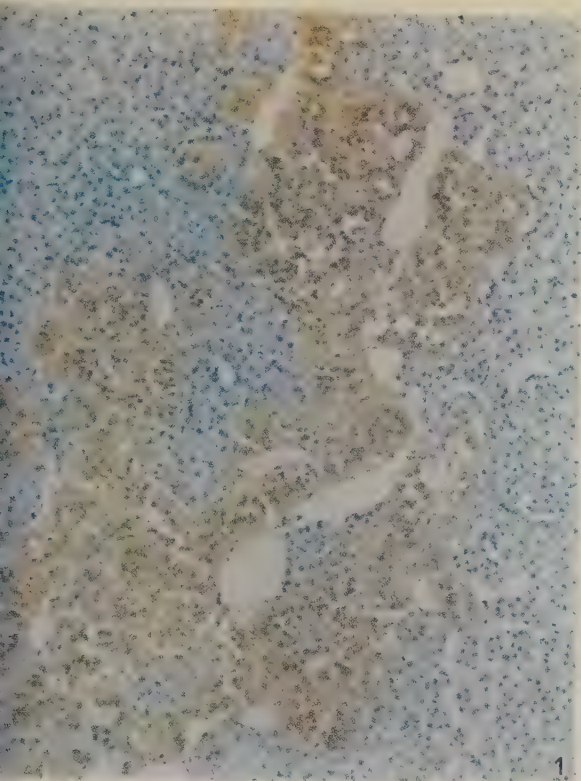
It is suggested that a chrome-adrenochrome is produced.

I wish to thank Prof. A. Durward for advice and encouragement; Dr W. K. J. Walls who has produced the photographic plates, and Mr R. Adkin for his assistance in the preparation of the histological material.

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#### EXPLANATION OF PLATE

- Fig. 1. Adrenal gland of a 4-week-old rabbit fixed by perfusion in formol-dichromate, stained with Ehrlich's haematoxylin. Medullary cells yellow-brown, cortex blue.  $\times 120$ .
- Fig. 2. Adrenal gland of an 8-week-old rabbit fixed by immersion in formol-dichromate, stained with Ehrlich's haematoxylin. Medulla in the centre of the field, cortex at the periphery. A well-marked chromaffin reaction is present in the venous sinuses but is very faint elsewhere in the medulla.  $\times 100$ .
- Fig. 3. Adrenal gland of an adult rabbit, fixed in formol-dichromate, stained with Giemsa. Medullary cells greenish blue, cortex violet. A few red blood corpuscles (red) are present in the central vein.  $\times 90$ .



# MUSCULAR CONTROL OF THE ARCHES OF THE FOOT IN STANDING: AN ELECTROMYOGRAPHIC ASSESSMENT

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The normal adult human foot is arched in coronal and sagittal planes, and this arched form persists to a greater or lesser degree even when the foot supports part of the body weight in standing. The mechanism by which this is achieved is controversial and the theories which have been advanced can be regarded as variations of two general views. On the one hand, it is held that the arches are maintained by the postural contraction of muscles, and on the other, that the foot is entirely supported by the passive strength of its own tissues.

The present investigation was undertaken to study the activity of those muscles of the leg and of the foot which might maintain, or help to maintain, the arches of the foot during standing. In some of the previous studies on this subject the activity of the muscles has been assessed by palpation (Jones, 1941; Keith, 1929), but this method has serious limitations. It is manifestly unsuitable for detecting minor grades of muscular activity, and moreover, it takes no account of the degree of tension in the tissues, such as deep fascia, which overlie the muscles. However, it is now generally accepted that the contraction of a muscle fibre is invariably associated with a change in electrical potential (action potential) in and around the fibre, and conversely, that these electrical changes are absent when the muscle fibre is at rest (Adrian, 1925; Lindsley, 1935; Weddell, Feinstein & Pattle, 1944). It was considered probable, therefore, that a more accurate assessment of the activity of the muscles of the leg and foot during standing would be achieved by studying the electrical changes occurring in those muscles by means of the electromyograph.

The mechanics of the foot during standing are related to the load which it has to support and to the duration of that load, and both these factors are dependent on the rhythmic nature of standing itself. The act of standing has been described recently elsewhere (Smith, 1953) and the following points are pertinent to the present investigation.

First, standing is not a continuously immobile act. On the contrary it is one in which short periods of almost complete immobility of the trunk and lower limbs—which may be termed the static phases—are punctuated by brief movements. In most individuals the average duration of the static phase of standing is about thirty seconds. Secondly, the postures during successive static phases in one individual may be of similar or different form. A posture may be of a symmetrical or an asymmetrical type. In the symmetrical posture (Text-fig. 1) the limbs and trunk are symmetrically disposed about the median plane, and each foot supports approximately half of body weight. In the asymmetrical postures, body weight is supported almost entirely on one lower limb while the other assists in maintaining balance. Weight may be carried predominantly by the left foot or the right and there are

therefore two varieties of this posture which are mirror images of each other: the left-footed asymmetrical posture is shown in Text-fig. 1. It is characteristic of the asymmetrical postures that in the balancing limb the knee is slightly flexed, and the foot is placed antero-lateral to its partner, and as a result, the pelvis is tilted



Text-fig. 1. The postures of normal standing. (*A*) illustrates the symmetrical posture in which each foot supports half of body weight. (*B*) illustrates the left-footed asymmetrical posture, in which the weight of the body is carried almost entirely by the left foot.

downwards and the vertebral column is convex on that side. In 1810 static phases observed in the investigation quoted above, one of the two asymmetrical postures occurred almost four times as often as did the symmetrical form.

Thus the act of standing has two alternating phases. During the brief active phases by which the posture may be altered, the foot is certainly under muscular control, and therefore this study has been limited to an investigation of the supporting mechanism of the foot during the intervening static phases. The stress which affects the foot during these phases depends on the posture which is adopted; if the posture is symmetrical each foot bears half of the body weight, whereas if it is asymmetrical, one foot carries almost the whole weight of the body and the other is practically free of any burden.

## METHOD

The electromyograph consists essentially of two electrodes, placed in, or close to, the muscle under examination and connected through an amplifier to a recording instrument, which in this case was a cathode-ray oscilloscope. The changes in potential occurring in or around the muscle cause deflexions of the oscilloscope beam, and these deflexions can be seen on a fluorescent screen or recorded on moving photographic paper.

In the experiments reported below, the amplifier was a three stage capacity-coupled model, with balanced input for use with surface electrodes, the rejection ratio being approximately 40,000:1. The frequency response was level from 30 to 25000 cyc./sec., and was 50 % down at 18 and 3000 cyc./sec. The recording instrument was a Cossor 1049 double-beam oscilloscope, and except when the contrary is stated, the upper beam traced the recording from the muscles under examination, while the lower beam was used as a time tracing, and showed the regular 50 cyc./sec. oscillations of the mains. The tracings shown were made on Ilford BP1 recording paper. Surface electrodes were used, and the subject was earthed by a large indifferent electrode placed on the skin nearby. The electrodes were polished steel pins,  $\frac{1}{8}$  in. in diameter, embedded 1 in. apart in a piece of Perspex plate. The ends of the pins which lay in contact with the skin were expanded and hollowed out so that the contact surface was cup-shaped. The skin on which the electrodes were to be placed was first washed with soap and hot water and then with methylated spirit. Cambridge electrode jelly was rubbed into the site until a distinct erythema was produced, after which the jelly was removed and the skin dried. The cups of the electrodes, having been filled with electrode jelly, were applied to the skin and held in place by an elastic strap of adjustable length.

Surface electrodes of this type were used in preference to the concentric needle electrodes introduced by Adrian & Bronk (1929). Needle electrodes, although of great value in the examination of small portions of a muscle—the purpose for which they were designed—are quite unsuitable for determining the absence of activity from a large area of muscle at any given time. They also have the disadvantage of causing some pain and apprehension in the subject, and thus render difficult the assumption of a completely natural attitude. In contrast, surface electrodes cause no discomfort to the subject and no restriction in the adoption of a natural attitude.

Adrian (1925) and Denny-Brown (1949) have shown that surface electrodes record the electrical changes from a comparatively large field. Of the muscles which were examined in the present investigation, the deep posterior crural muscles lie farthest from the skin surface. It has been demonstrated by the experiment described below that activity in this group of muscles was adequately recorded by the type of surface electrodes already described.

The subject was seated, and one thigh was supported so that the knee joint was almost fully flexed and the leg hung vertically downwards (Pl. 1, fig. 2). Electrodes were applied to the skin over the lower part of the medial head of the gastrocnemius muscle in the supported limb, and the subject carried out a series of movements which produced the recording in Pl. 1, fig. 1. He first plantar-flexed the relevant ankle joint against resistance, producing the part of the record labelled *A*. He then



relaxed the whole limb as completely as possible and the corresponding part of the record is labelled *B*. Thereafter the ankle joint was moved into full plantar-flexion (record segment *C*), and subsequently maintained in that position as strongly as possible (record segment *D*).

While the ankle joint was plantar-flexed against resistance the gastrocnemius and soleus muscles were tense on palpation, and it is considered that the corresponding part of the record is mainly due to their activity. During relaxation of the limb no activity was recorded. The remaining two parts of the record (*C* and *D*), which were produced during the movement of plantar-flexion of the ankle joint, and while the ankle joint was maintained in full plantar-flexion, differ in form. The former closely resembles the record of plantar-flexion against resistance (*A*), and it is believed that it was similarly produced by the activity of the gastrocnemius and soleus muscles. In the latter (*D*), however, although the frequency of the deflexions is unchanged, their amplitude is much reduced and the direction of the larger deflexions is predominantly downwards. This change in form is regarded as an indication that the region of muscular activity shifted away from the electrodes and acquired a new orientation to them.

Cyriax (1917) and Haines (1934) have both observed that when the knee joint is in a flexed position, plantar-flexion of the ankle joint is initiated by the gastrocnemius and soleus muscles, but that towards the end of the movement these muscles become flaccid and the tendo-calcaneus completely slack; this change in the tension of the tendon is illustrated in Pl. 1, figs. 2 and 3. It has been suggested by Cyriax and Haines that the terminal phase of the movement is effected, and the final position is maintained, by the deep posterior crural and peroneal muscles. There seems little doubt that this change in the motivation of the movement is reflected in the alteration of the record between parts *C* and *D* in Pl. 1, fig. 1, and that the deflexions in part *D* are due to the activity of the deep posterior crural muscles.

Using the apparatus described above, the activity of the anterior crural, posterior crural, peroneal and short plantar groups of muscles has been examined during the immobile periods of standing. Six subjects were so examined: other subjects were rejected because it was obvious that even with practice they were unable to settle into relaxed and comfortable attitudes under the conditions of the experiment.

## OBSERVATIONS

### *Examination of the anterior crural group of muscles in standing*

Surface electrodes were applied to the anterior aspect of the leg and their exact position was varied in successive experiments so that every part of the anterior crural group of muscles lay within the effective range of the electrodes on at least one occasion. When the subject stood in an asymmetrical posture the electrodes were applied to the supporting limb, whereas if he stood symmetrically two recordings were made: the electrodes were applied to the left leg during the one recording and to the right leg during the other. In each experiment the standing subject first dorsiflexed the relevant ankle joint and then relaxed into a comfortable posture either symmetrical or asymmetrical in form. The form of the resulting electromyograms was independent of the type of posture, of the position of the electrodes within the limits already stated, and of the duration of the recording.

An example of the tracings is shown in Pl. 2, fig. 4. That part of the tracing associated with dorsiflexion of the ankle joint extends as far as the arrow; it is grossly disturbed and is a typical record of strong voluntary muscular action. The remainder of the electromyogram was recorded while the subject was standing comfortably. It shows minute deflexions which from their regular form and frequency are certainly due to interference from the mains. Apart from these the tracing is undisturbed for a period of  $3\frac{1}{2}$  sec. The quiescence of the relevant parts of these records indicates that the anterior crural muscles are inactive during the static phases of standing.

#### *Examination of the posterior crural muscles in standing*

In this group of experiments each subject first stood on tiptoe and then settled into a comfortable attitude: in some the posture was symmetrical and in others asymmetrical. The electrodes were applied to the skin on the posterior aspect of the leg—the supporting leg if the subject proposed to stand asymmetrically, and to the right and left legs during successive recordings if he meant to stand symmetrically—and in the several experiments they were moved to various positions between the knee and ankle joints so that all fibres of the muscle group lay, at least on one occasion, within their range.

The form of the records which were thus obtained was independent of the type of posture which the subject adopted—and therefore of the load on the foot—but was related to the position of the electrodes. Pl. 2, fig. 5, is a typical example of the recordings obtained when the electrodes lay over the gastrocnemius. The part of the tracing that extends to the arrow was recorded while the subject stood on tiptoe and strong contraction of the posterior crural muscles is indicated. The electromyogram between the first and second arrows was made while the subject was standing comfortably: groups of deflexions (underlined) are separated by undisturbed parts of the tracings, and this periodicity indicates an alternation of activity and rest in the posterior crural muscles. When the electrodes were moved distally away from the gastrocnemius the deflexions became progressively smaller in amplitude and disappeared entirely before the ankle joint was reached.

Two problems arise from these observations, namely the location of the bursts of activity within the posterior crural group, and secondly, the relationship of that activity to the stability of the ankle joint on the one hand and the stability of the foot on the other.

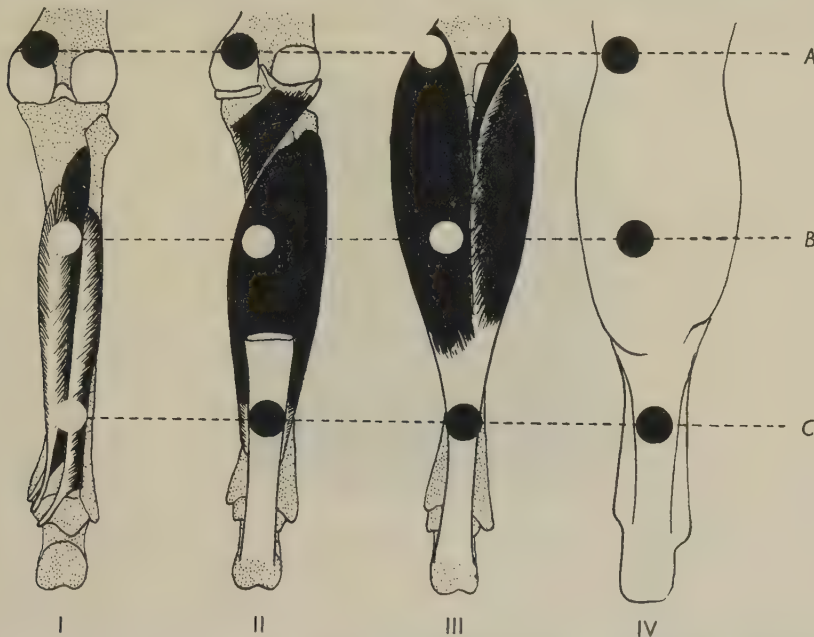
#### *The location of activity in the posterior crural muscles during standing*

An examination with surface electrodes is incapable of determining with absolute certainty the location of activity within a muscle group. Nevertheless, it is considered that some observations made by this method are significant.

The relationship of surface electrodes to the individual muscles of the posterior crural group varies with the position of the electrodes. Text-fig. 2 is a diagrammatic representation of the posterior crural muscles: the deep muscles are shown in I, the soleus and popliteus muscles in II, the gastrocnemius and plantaris in III, and the skin outline of the leg and foot in IV.

Electrodes placed in position *A* would be closely related to the gastrocnemius but remote from other muscles. Placed in position *B* they would be closely related to

the gastrocnemius, soleus and the deep posterior crural muscles. In position *C* electrodes would be closely related to the soleus and the deep posterior crural muscles but would be remote from the gastrocnemius. It is appreciated that there is considerable variation in the proximo-distal extent of these muscles, but from my own observations the variations are never sufficient to invalidate these relationships.



Text-fig. 2. The posterior crural muscles. The circles indicate the positions of the electrodes referred to in the text.

It has been observed that when the electrodes are applied in position *B* while the subject is standing comfortably, an electromyogram is produced which is similar to that in Pl. 2, fig. 5. Movement of the electrodes distally results in a progressive diminution in the amplitude of the recording and all deflexions disappear when position *C* is reached. Movement of the electrodes proximally from position *B* to position *A* brings about no change in the pattern of the electromyogram. Thus the amplitude of the electromyogram is dependent on the relationship of the electrodes to the gastrocnemius and is independent of their relationship to the other muscles of the group. It is consequently believed that the activity shown in the electromyogram in Pl. 2, fig. 5, is mainly located in the gastrocnemius muscle.

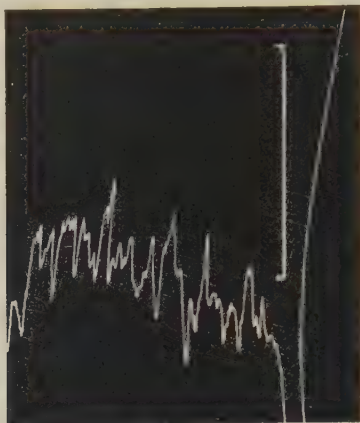
*The relationships of the activity of the posterior crural muscles to the movements of the ankle joint during standing*

It is well known that throughout any period of standing there is a continuous slight antero-posterior oscillation of the body over the feet, and that the movement occurs mainly at the ankle joint. The anterior and posterior movements of the upper end of the tibia in this general swaying are illustrated by the upward and downward



deviations of the kymograph tracing in Text-fig. 3. The duration of this tracing was 1 min.: the bracket denotes the deflexion produced by a movement of the upper end of the tibia of 1.5 cm.

The intermittent activity of the posterior crural muscles demonstrated in Pl. 2, fig. 5, is related, in the dimension of time, to these antero-posterior movements of the body. To demonstrate this relationship, the lower beam of the oscilloscope—used in the other experiments as a time tracing—was made to record the varying

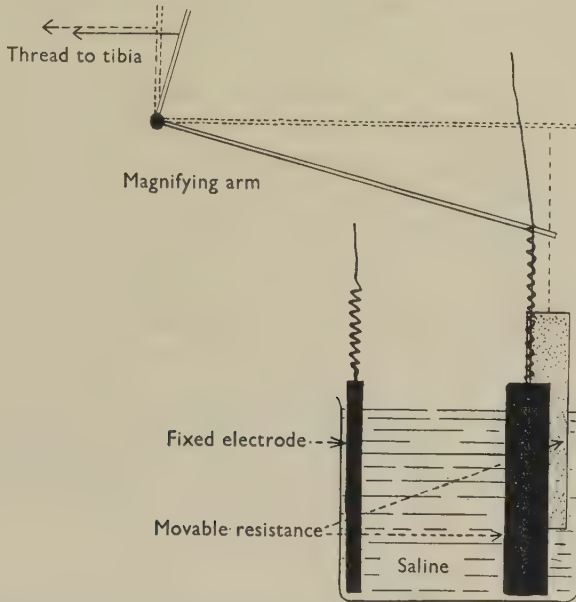


Text-fig. 3. Kymograph illustrating the antero-posterior swaying of the body which occurs during standing.

position of the body in the following way. The frequency of the alternating current controlling this beam was increased from 50 to 300 cyc./sec. With this frequency, the individual deflexions of the beam became fused on the recording into an 'envelope' tracing, e.g. the lower tracing in each strip in Pl. 2, fig. 6. The variable resistance illustrated in Text-fig. 4 was then introduced into the circuit. It consisted of a fixed electrode and a movable resistance immersed in saline. The movable resistance was connected through a magnifying arm to the upper end of the tibia of the subject under examination, so that it progressively emerged from the saline as the tibia moved backwards and became increasingly immersed as the tibia swayed forwards. With this arrangement the resistance of the circuit varied inversely with the length of the movable resistance which was immersed. In other words, the resistance of the circuit increased as the body swayed backwards and decreased as it swayed forwards. The amplitude of the deflexions of the oscilloscope beam produced by the alternating current, and therefore the width of the envelope tracing, vary with the resistance of the circuit. Therefore in this experiment the envelope tracing contracted as the body swayed forwards and expanded as it swayed backwards.

Pl. 2, fig. 6, is a recording taken from a standing subject with surface electrodes over the lower part of the gastrocnemius. Each strip shows two tracings; the upper is the electromyogram of the posterior crural muscles, and the lower is the envelope tracing which indicates the antero-posterior movements of the body. The two tracings are automatically synchronized. Each burst of muscular activity begins as the body

starts to sway forwards, i.e. as the lower tracing begins to contract, and continues until it has passed the anterior limit of its oscillation and is swaying backwards, i.e. until the lower tracing has reached its minimum width and is expanding. The muscular activity then stops, and during the remainder of the backward swing, the electromyogram is undisturbed. Hellebrandt & Braun (1939) have shown that the antero-posterior movements of the body during standing do not represent a bilaterally equal oscillation about a mean position of stability. On the contrary, the centre of gravity of the body lies anterior to the axis of rotation of the ankle joint throughout



Text-fig. 4. The variable resistance. Movements of the upper end of the tibia are associated with either an increase or a decrease in the length of the movable resistance which is immersed in saline.

these movements. It therefore appears, from Pl. 2, fig. 6, that as the body sways forwards, it is gradually decelerated and finally arrested by the posterior crural muscles. The same muscles then draw the body backwards towards, but not as far as, the posterior limit of its oscillation, and the last part of the movement is due to the body's momentum alone. When this momentum is expended, the centre of gravity is still anterior to the axis of the ankle joint, and so the body begins to sway forwards again and the whole cycle is repeated. Joseph & Nightingale (1952) have suggested that the activity of the posterior crural muscles is continuous and of uniform intensity throughout standing. This seems unlikely in view of the antero-posterior swaying of the body demonstrated in Text-fig. 3, and it is suggested that their results may be due to their recordings being of insufficient length or to a failure on the part of their subjects to relax.

*The effect of the activity of the posterior crural muscles on the foot during standing*

It has been noted that activity in these muscles during standing is most probably limited to the gastrocnemius. If this deduction is correct, the posterior crural muscles can have no effect on the calcaneo-cuboid joint or the joints of the forefoot, but, because the gastrocnemius muscle is functionally associated with the subtalar joint, it is possible that its activity might help to resist, though only intermittently, the tendency of body weight to cause eversion at that joint.

The fact that the degree of activity in the gastrocnemius during standing is independent of the attitude, and therefore of the load on the foot (*vide supra*), strongly suggests that the muscle does not help to support the foot, and this is confirmed by the observation described below.

Pl. 3, fig. 7, shows the last strip of Pl. 2, fig. 5, and a continuation of the same electromyograph. As far as the arrow the subject was standing comfortably and the record shows one of the periods of muscular activity which have just been described. At the point on the record indicated by the arrow, the subject, while maintaining the same attitude, placed the fingers of one hand on the back of a chair. Beyond this point, the electromyogram indicates complete inactivity of the posterior crural muscles, except for one isolated action potential in the third strip. Obviously the light pressure of the fingers on the chair was sufficient to counteract the tendency of the body to sway forwards, and the posterior crural muscles consequently relaxed. Despite this muscular inactivity, the foot was still supporting virtually the same load, and there was no apparent change in its form. It is consequently believed that the intermittent activity of the posterior tibial muscles which controls the position of the ankle joint during standing, takes little if any part in maintaining the form of the foot during the static phases of that act.

*Examination of the short plantar muscles in standing*

Surface electrodes were applied to the central part of the sole of the foot, and the subject stood on a platform in which a gap had been cut corresponding to the non-weight bearing area of the sole. In this way it was possible for the subject to adopt a normal attitude without causing pressure on the electrodes. It was found by measurements made on sections of the foot and leg that with this arrangement the deepest fibres of the short plantar muscles were no farther from the electrodes than were the deep posterior crural muscles during the experiment illustrated in Pl. 1, fig. 1. Moreover, the thick plantar fascia intervening between the electrodes and the muscles has been shown by Weddell and his associates (1944) to form no barrier to the spread of an electrical field. On these grounds it is considered that during these experiments all the short plantar muscles lay within the effective range of the electrodes.

In each instance the standing subject first flexed his toes to produce marked deflexions on the recording, and then settled into a comfortable posture, symmetrical in some cases and asymmetrical in others.

Pl. 3, fig. 8, is a typical recording. It begins with the pronounced deflexions associated with the flexion of the toes. At the point indicated by the first arrow the subject adopted a symmetrical attitude, and after an interval of about 2 sec.



(between the first and second arrows) of diminishing activity the tracing remains undisturbed for a period of nearly 5 sec. During the recording of this electromyogram the subject was standing symmetrically, but essentially similar records were obtained during an asymmetrical posture.

Thus typically during each immobile period of standing, in either the symmetrical or the asymmetrical posture, the short plantar muscles relax after the first 1 or 2 sec. and are subsequently inactive.

*Examination of the peroneus longus and brevis muscles in standing*

These muscles cannot be examined as an isolated group with surface electrodes, because of their close proximity to both the anterior and posterior crural groups. Despite this difficulty, however, it is considered that the activity of the peroneal muscles during standing can be assessed indirectly.

While the anterior crural muscles were being examined, it was found that when the electrodes were placed over the extensor digitorum longus muscle, that is within about  $1\frac{1}{2}$  cm. of the peroneus longus and brevis, no action potentials were recorded during standing. Considering the proximity of the electrodes to the peroneal muscles it seems reasonable to assume that the muscles were inactive.

When the electrodes were placed directly over the peroneus longus, the electromyogram obtained during standing was similar to the recording derived from the posterior tibial muscles. The activity had the same periodicity, but the average amplitude of the deflexions was much less. These facts strongly suggest that the potential changes which were recorded, were in fact located in the posterior tibial muscles and not in those of the peroneal group.

On these grounds it is considered that the peroneus longus and brevis muscles are inactive during each static phase of standing.

CONCLUSIONS

The act of standing consists of a series of practically static phases of an average duration of 30 sec., which alternate with brief phases of movement. It has been shown that during the greater part of each static phase of standing, all the muscles directly associated with the foot, with the exception of the gastrocnemius, are inactive. The gastrocnemius contracts intermittently throughout each static phase, but the observations which have been made indicate that this activity is concerned with the control of the antero-posterior sway of the body and appears to have little effect on the foot.

It is therefore believed that during the greater part of each static phase of standing the arched form of the foot is maintained against the force of body weight by a mechanism involving the passive strength of the tissues of the part. The bones, ligaments and fascial bands probably play the major role in this mechanism, but the observations which have been reported do not exclude muscles as passive structures playing a minor part. This conclusion seems to be in keeping with recent observations by Seyffarth (1940, 1941), Floyd & Silver (1951*a, b*) and Clemmesen (1951) on other parts—observations which led Clemmesen to postulate, '...that the postural reflex tone subsides or is suppressed for a while in certain circumstances'.

Thus throughout the act of standing, as the person alternates between movement

and immobility, the mechanism maintaining the arch of the foot changes from the muscular to the osteo-ligamentous, and each mechanism acts continuously for a comparatively short time. The normal function of both mechanisms is essential to normal standing but they act alternately, not synchronously.

#### SUMMARY

1. The activity of the anterior crural, posterior crural, peroneal and short plantar muscles during the immobile periods of standing has been examined by means of the electromyograph.

2. Activity was noted in the posterior crural group of muscles, and it is suggested that this was located mainly in the gastrocnemius. It is believed that this activity is concerned with the antero-posterior swaying of the body and that it has no demonstrable effect on the form of the foot.

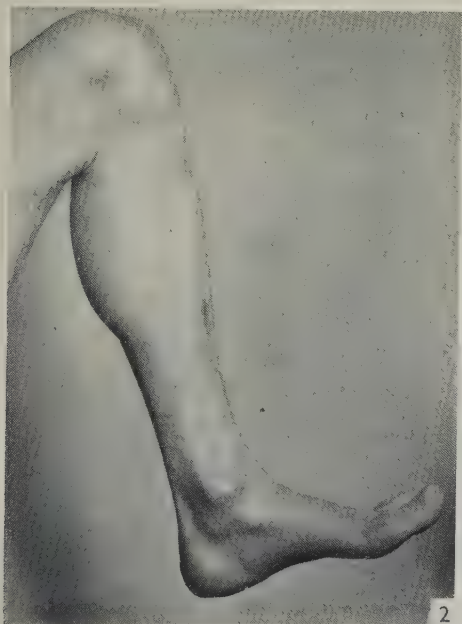
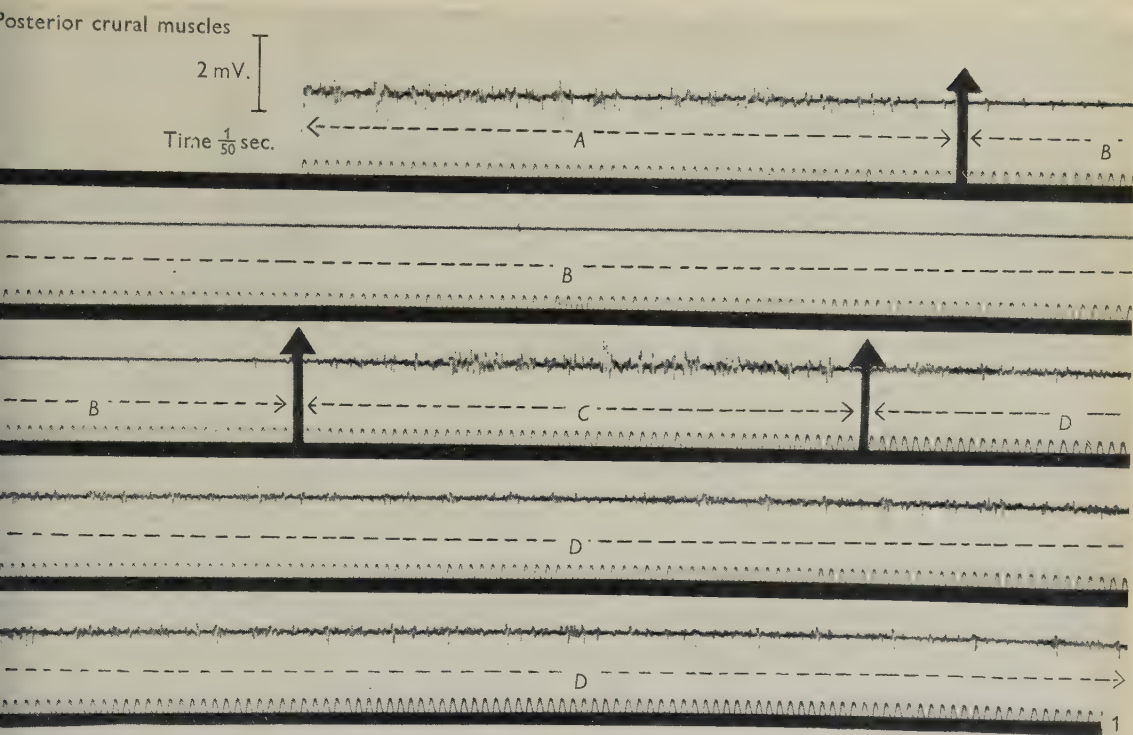
3. The other muscle groups were inactive during the static phases of standing.

4. It is suggested that during standing, as movement alternates with immobility, so the mechanism supporting the arches of the foot changes from the muscular to the osteo-ligamentous.

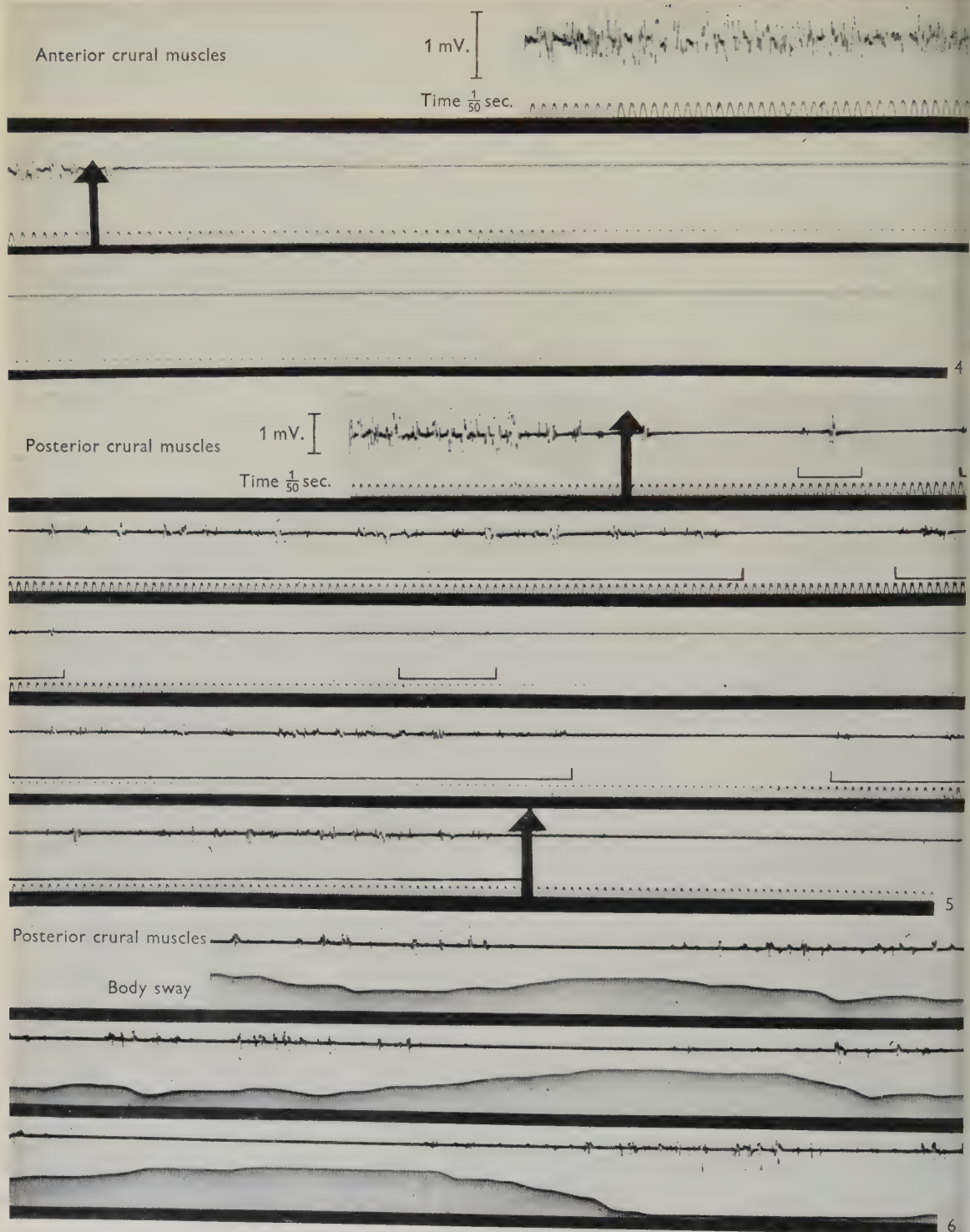
I wish to thank Prof. R. Walmsley and Prof. A. E. Ritchie for the help and advice they gave me during this investigation.

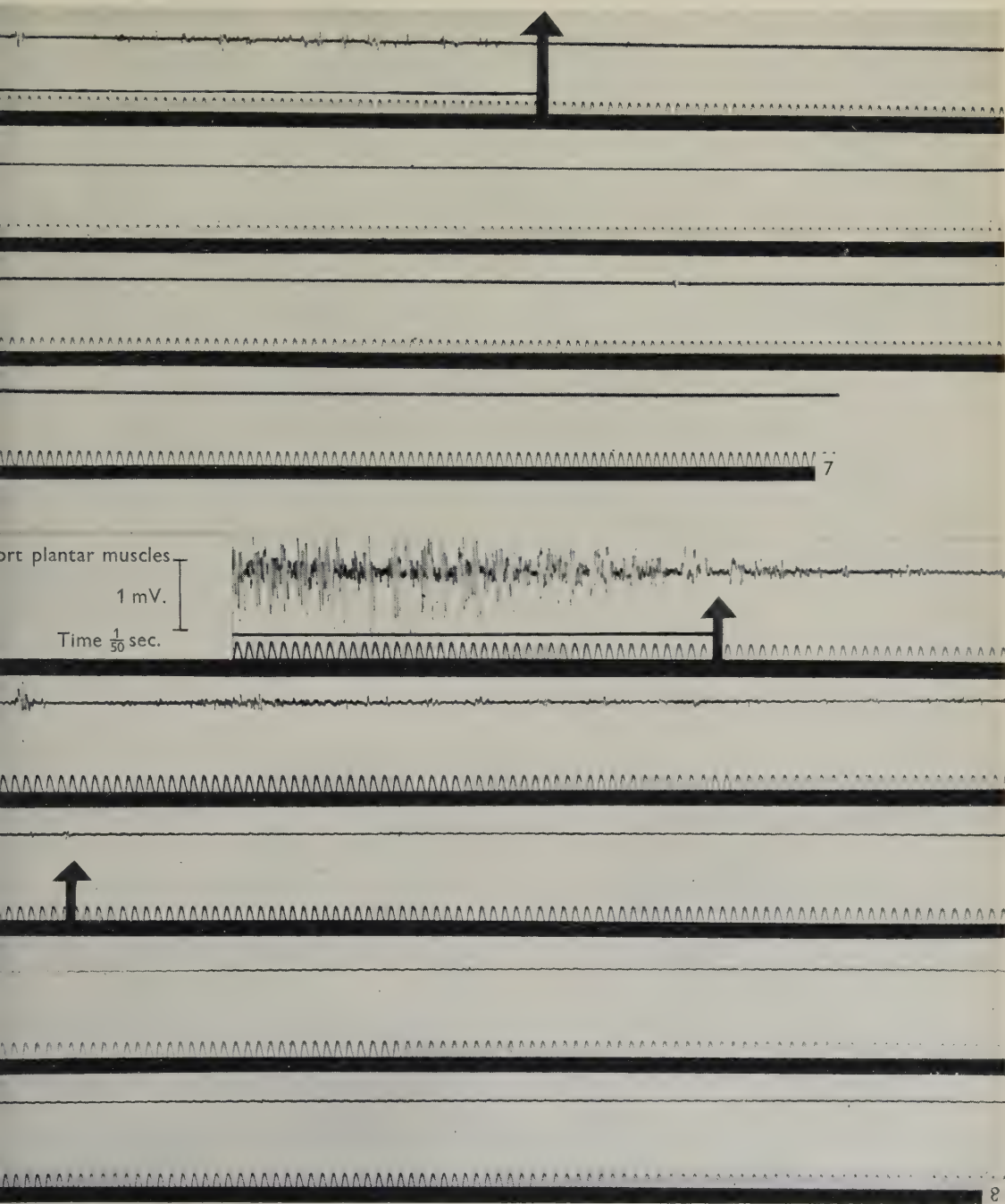
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WITH—MUSCULAR CONTROL OF THE ARCHES OF THE FOOT IN STANDING





EXPLANATION OF PLATES

PLATE 1

- Fig. 1. The activity of the posterior crural muscles. Time tracing  $\frac{1}{50}$  sec. A deflexion of 5 mm. indicates a change in potential of 1 mV.
- Fig. 2. The position of the lower limb at the beginning of segment C of the recording in Pl. 1, fig. 1.
- Fig. 3. The position of the lower limb during segment D of the recording in Pl. 1, fig. 1. Note the slackness of the tendo calcaneus.

PLATE 2

- Fig. 4. The activity of the anterior crural muscles during dorsiflexion of the ankle and (beyond the arrow) during standing. Time tracing  $\frac{1}{50}$  sec. A deflexion of 10 mm. indicates a potential change of 1 mV.
- Fig. 5. The activity of the posterior crural muscles. As far as the first arrow the subject was standing on tiptoe, and between the first and second arrows he was standing asymmetrically.
- Fig. 6. Upper tracing in each strip is the electromyogram of the posterior crural muscles. Expansion and contraction of the lower envelope tracing indicates backward and forward movements of the body respectively.

PLATE 3

- Fig. 7. Continuation of the electromyogram in Pl. 2, fig. 5, showing the activity of the posterior crural muscles during standing, and (beyond the arrow) during standing with support.
- Fig. 8. Electromyogram of the short plantar muscles. As far as the first arrow the subject was standing and at the same time flexing his toes. Beyond the first arrow he was standing comfortably. Time tracing  $\frac{1}{50}$  sec. A deflexion of 10 mm. indicates a potential change of 1 mV.

## THE VASCULARITY OF THE CEREBRAL CORTEX IN NORMAL AND CRETINOUS RATS

By J. T. EAYRS

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Vascular degeneration, followed by sclerosis, is frequently associated with chronic thyroid deficiency. The primary lesion is believed to be in the capillary endothelium of the vasa vasorum (Kountz, 1951), and has been attributed to hypercholesterolaemia and disturbed fat metabolism (Leary, 1941). Hueper (1941) has suggested that stagnant anoxia is also a causative factor, a suggestion which accords with the observation that the circulation time is prolonged in myxoedema (Lange, 1944; McGavack & Schwimmer, 1944). Thyroid deficiency is also associated with a reduction in the number of cutaneous capillaries (Zondek, Michael & Kaatz, 1941).

These considerations suggest that changes in the cerebral circulation might help to account for the retarded mental development and impaired cerebral function which characterize the cretin. There has been little opportunity for studying this possibility in man, since, as Benda (1947) has pointed out, the brains of relatively few young cretins come to autopsy. His own view is that the changes in cerebral vascularity resemble those observed in the brains of mongoloid idiots: the vessels are less numerous than in normal individuals, the capillaries are enlarged, and there are signs of vascular stasis and oedema.

The hypothyroid state can, however, be produced in laboratory animals by giving methyl thiouracil, and the present paper reports the results of an experiment designed to elucidate the nature of the changes that occur in the vascularity of the cerebral cortex of rats made cretinous in this way.

### MATERIALS AND METHODS

#### *Preparation of tissues*

Eight litters of young rats were used in this study. The day after they were born half of the rats of each litter, in equal numbers of each sex, were injected with 0.05 ml. of a 4% suspension of methyl thiouracil in a 2% solution of sodium alginate in physiological saline. The remainder were injected with the sodium alginate solution alone. Similar injections were given daily for the next 4 days, after which the dose was increased to 0.10 ml. for a further 10 days, and again to 0.20 ml. until the animals were killed. In order that the slower rate of growth and reduced activity of rats given methyl thiouracil should not place them at a nutritional disadvantage when competing for food with the control animals, half of one litter was interchanged with half of another litter born on the same day so that all the experimental animals of this pair of litters were raised by one of the two does and all controls by the other. Mortality among the animals given methyl thiouracil was high after the 18th day. Whenever one of these died a litter-mate of the same sex was removed from the control litter.

When the rats were 24 days old the surviving animals were anaesthetized with

ether and perfused with saline at 37° C. (see Eayrs, 1950). The perfusion was immediately followed by an injection of carmine gelatin, after which the head of the animal was placed in ice-cold saline until the gelatin had set. The brain was then removed, fixed for 6 hr. in Bouin's aqueous solution, transferred to 70% alcohol for 24 hr. and subsequently embedded in paraffin. The brains were sectioned at about 10 $\mu$  through the sensorimotor cortex in the plane previously studied by Eayrs & Taylor (1951), and the sections lightly stained with toluidin blue. Twelve pairs of brains were successfully prepared in this way.

#### *Quantitative estimation*

The vascularity of the cortex of each rat was estimated by making drawings under the projection microscope ( $\times 450$ ) of all blood vessels in eight microscopic fields, randomly selected from within the sectors shown in Fig. 1. The proportion of each field occupied by blood vessels was then estimated planimetrically.

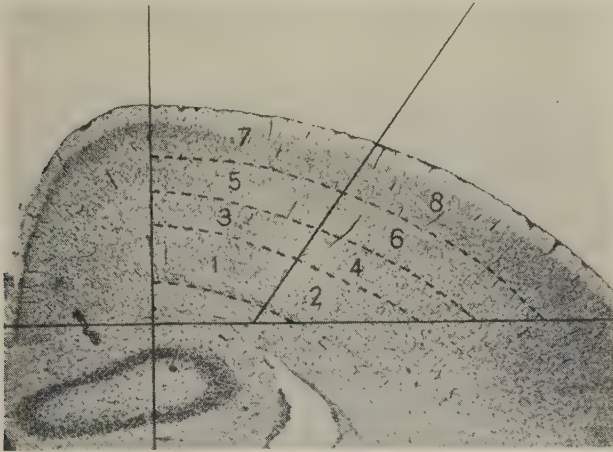


Fig. 1. Coronal section through the sensorimotor cortex of the rat showing the zones within which measurements of vascularity were made. Sectors numbered 1/2 and 3/4 correspond respectively with layers 6 and 5; sectors 5/6 include the deeper part of layer 3 with layer 4. Layer 1 was not measured. ( $\times 19$ .)

A prominent feature of the cerebral circulation is that the capillary network tends to empty directly into large collecting venules (Campbell, 1938) which are present in all regions of the cortex. The fortuitous inclusion of one or more of these large vessels in some, but not in all, of the selected fields would, by increasing the variance of the measurements, tend to mask any changes in the dimensions of the capillary bed which might have resulted from the experimental treatment. Craigie (1938) estimated the mean diameter of capillaries in the cerebral cortex of the albino rat as 2.9 $\mu$  so that, since there were no precise histological criteria for differentiating large capillaries from small venules, a diameter of 5 $\mu$  was arbitrarily selected as the upper limit of size for capillaries. Three types of measurements were therefore made: the total area within each microscopic field occupied by blood vessels of all sizes; the



area occupied by vessels exceeding  $5\mu$  in diameter (large vessels); and the area occupied by vessels of  $5\mu$  or less in diameter (capillaries). The number of fragments of blood vessels present in each field was counted and classified in the same way.

### *Sources of error*

#### *(i) Errors arising during preparation of tissues*

Several techniques for demonstrating the vascular bed were tried but none was completely successful. The injection of indian ink, and the method described by Pickworth (1934), which makes use of the presence of red blood corpuscles in the vessels, did not give sufficient continuity of outline to enable accurate drawings of capillaries to be made. The plasma stain advocated by Campbell (1938) proved too capricious in use, the animals often dying before an adequate concentration of the dye had been injected into the blood stream. In the end trial and error suggested that the carmine-gelatin method was the best available. There can be no certainty that the whole vascular bed was in every case completely injected by this method, but any less successful injections are likely to have been divided equally between the experimental and control rats.

#### *(ii) Errors arising during drawing and measurement*

Unless the whole thickness of the section falls within the depth of focus of the microscope objective, the ends of some blood vessels, where they pass out of focus, present a blurred outline which cannot be drawn accurately. On the other hand, if the sections are very thin, the capillary fragments are so small that the error of planimetry becomes disproportionately high. Accordingly, each vessel was drawn by focusing down through the thickness of the section and tracing its outline as its wall came fully into focus. This procedure introduces a small systematic error due to foreshortening, each vessel being drawn rather shorter than its true length. Since, however, the object of the estimations was to compare the vascularity of two similarly constituted tissues and not to provide absolute data, this source of error is hardly likely to vitiate the results of the comparison.

Sample checks of the non-systematic error introduced by inaccuracies in drawing and planimetry showed that in no case did a repeated measurement differ by more than 5% from the original.

#### *(iii) Errors resulting from differences in section thickness*

It was found during the estimations that experimental and control sections were not always of exactly the same thickness. While these differences were generally small it was thought necessary to apply an appropriate correction. If it is assumed that the vascular network is randomly arranged in three dimensions, the number of capillary fragments will be independent of, and the projected area of the vascular bed proportional to the thickness of the section. On this basis measurements were standardized to a section thickness of  $10\mu$ . Some difficulty was presented by the large vessels, which tend to be radially orientated in relation to the cortical surface and some of which were wider than the thickness of the section. Both these factors introduce a small error into the adjusted data, and while this error is unlikely to

bias the comparison between experimental and control tissue, the statistics relating to the large vessels must be regarded as somewhat less reliable than those for the small.

### *Statistical treatment of the data*

The data were treated statistically by the method of analysis of variance. Data representing (i) the number of fragments of blood vessels counted in a square millimetre of tissue, (ii) the percentage of cortical tissue occupied by blood vessels, and (iii) the mean size in square microns of the fragments counted were first analysed as a whole without reference to the laminae or region of the cortex in which the measurements were made. Sums of squares were taken out for the mean of the data, for the effect of differences due to treatment (i.e. the administration of methyl thiouracil) and for differences between pairs of experimental and control rats not due to this treatment. The residual sum of squares was used as an estimate of error to test whether the effect of thyroid deficiency was such as might be expected to fall outside the range of variation between normal individuals. The data were then rearranged to show, and to test the significance of, any differences in vascularity between the several cortical laminae and between the medial and lateral regions of the cortex.

## RESULTS

### *Changes in vascular pattern due to thyroidectomy*

Table 1 shows that while there were more large blood vessels (over  $5\mu$  in diameter) in the tissues of the thyroid-deficient animals than in those of the controls, there were significantly fewer capillaries. At the same time, the proportion of cortical tissue

Table 1. *Differences in cortical vascularity between normal and cretinous albino rats measured in terms of (a) mean number of fragments of blood vessels counted in  $1\text{ mm.}^2$  of tissue; (b) mean percentage of tissue occupied by blood vessels and (c) mean size of fragments of blood vessels ( $\mu^2$  projected area)*

Class of vessel		Normal	Cretinous	Difference	<i>t</i>	<i>P</i>
All vessels combined	<i>a</i>	$521.5 \pm 20.95^*$	$432.4 \pm 27.16$	$89.1 \pm 31.24$	3.382	<0.01
	<i>b</i>	$4.64 \pm 0.366$	$5.28 \pm 0.461$	$0.63 \pm 0.587$	1.562	0.2-0.1
	<i>c</i>	$92.3 \pm 9.66$	$129.9 \pm 16.37$	$37.7 \pm 19.03$	2.871	0.02-0.01
Vessels over $5\mu$ in diameter	<i>a</i>	$21.0 \pm 1.48$	$31.7 \pm 5.64$	$10.7 \pm 5.83$	2.168	0.1-0.05
	<i>b</i>	$1.43 \pm 0.292$	$2.18 \pm 0.393$	$0.74 \pm 0.489$	2.265	0.05-0.02
	<i>c</i>	$636.6 \pm 109.01$	$694.1 \pm 101.33$	$57.5 \pm 148.86$	Very small	
Vessels $5\mu$ and less in diameter	<i>a</i>	$500.5 \pm 21.89$	$400.8 \pm 26.10$	$99.7 \pm 24.05$	3.435	<0.01
	<i>b</i>	$3.21 \pm 0.112$	$3.10 \pm 0.155$	$0.11 \pm 0.191$	Very small	
	<i>c</i>	$65.7 \pm 3.72$	$81.1 \pm 7.05$	$15.5 \pm 7.97$	2.422	0.05-0.02

\* This, and the similarly quoted figures throughout the table, are the standard errors of the mean and are based in each case on twelve observations.

occupied by the vascular bed was approximately the same in the two groups, although the blood vessels in the experimental tissues tended to occupy more space than in the controls. This difference was almost entirely due to the fact that the large vessels were more numerous in the experimental tissues. On the other hand, the capillaries occupied almost the same relative volume in both experimental and control tissues, an increase in the mean size of the fragments in the thyroidectomized animals having compensated for the reduced numbers.

*Differences in vascularity between cortical laminae*

In the tissues of the normal animals there were marked and statistically significant differences in vascularity between the cortical laminae measured in terms both of the number of fragments of blood vessels contained in each layer and of the proportion of the cortical tissue they occupied (Figs. 2, 3). Measured from the pial surface

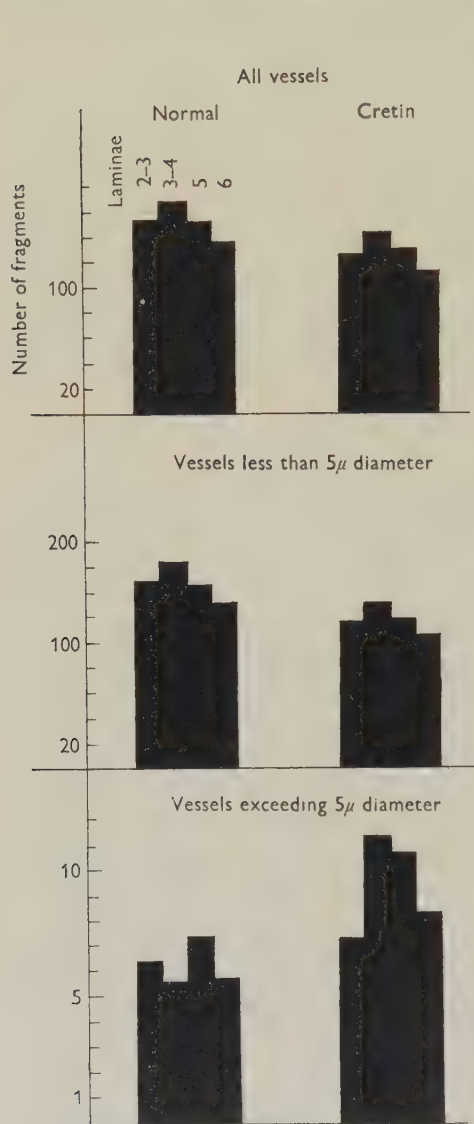


Fig. 2.

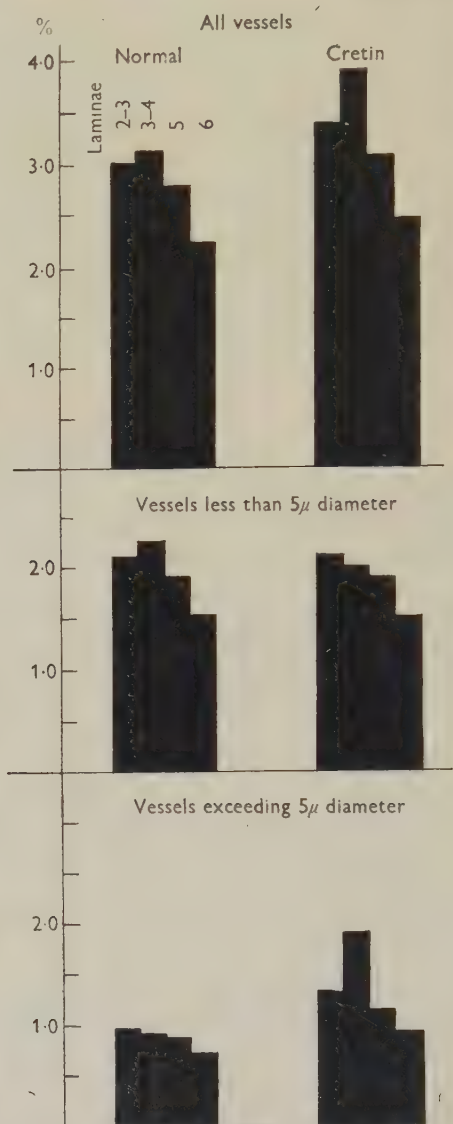


Fig. 3.

Fig. 2. Diagram showing the mean number of fragments of blood vessels at different levels in the cerebral cortex of normal and cretinous rats. Each histogram refers to a microscopic field of area 1 mm.<sup>2</sup>

Fig. 3. Diagram showing the percentage of cortical tissue occupied by blood vessels in different laminae in normal and cretinous rats.



of the cortex the gradient of density of the vascular bed initially increased but fell away towards the deeper strata. Layer 4 (with the adjacent parts of layer 3) was the most vascular and layer 6 the least (Figs. 2, 3). The number of capillaries in the granular and supra-granular layers combined was 11 % greater than in the infra-

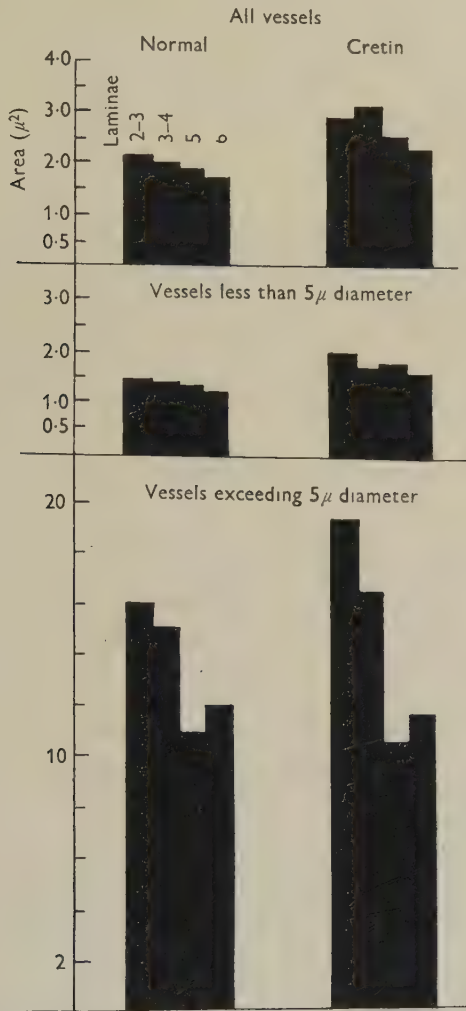


Fig. 4. Diagram showing the mean size of blood vessels in the different laminae of the cerebral cortex of normal and cretinous rats.

granular layers ( $P < 0.01$ ), but there was no corresponding difference in the relative numbers of the larger vessels (Fig. 2). The vascular bed (large vessels and capillaries alike) occupied 20 % less cortical tissue ( $P < 0.01$ ) in the deeper laminae than in the more superficial (Fig. 3), and likewise the mean size of the blood vessels was 17 % greater ( $P < 0.01$ ) in the granular and supra-granular laminae than in the infra-granular (Fig. 4).

The experimental treatment accentuated certain of these inter-laminar differences,

but did not otherwise change the normal pattern of cortical vascularity described above. One of its effects was a 70 % increase in the number of large vessels present in layers 3-5 ( $P=0.02-0.01$ ). This effect was most marked in the granular layers, where a considerable increase in the number of large vessels and of the proportion of cortical tissue which they occupied was offset by a reduction in capillarity (Fig. 2). Thyroid hypofunction also accentuated by a factor of 3 the difference in mean size between the blood vessels in the supra-granular and granular layers and those of the infra-granular layers, an effect which was almost entirely due to a significant increase in the mean size of large blood vessels contained in layers 2-4 (Fig. 4).

#### *Regional differences in the distribution of blood vessels*

In both normal and experimental tissues the total numbers of blood vessels present in the medial and lateral regions of the cortex did not differ markedly. On the other hand, in the lateral part of the cortex (2, 4, 6 and 8 on Fig. 1) which is more granular than the medial, the proportion of cortical tissue occupied by blood vessels was 15 % greater than in the medial ( $P<0.01$ ). This difference was due to the increased space taken up by large vessels and capillaries alike.

#### DISCUSSION

The mode of distribution of cortical blood vessels shown by the three measures used in the present experiment conforms closely with that described by Craigie (1921) on the basis of the total length of capillaries present in 1 mm.<sup>3</sup> of tissue. Although the experimental treatment did not alter this pattern of distribution, comparison of the experimental and control tissues shows that thyroid hypofunction is associated with two major changes in vascularity. In the first place, the vascular bed in the cortex of the cretinous rat is hypoplastic in the sense that it contains significantly fewer blood vessels in a standardized microscopic field than are present in the normal animal. Secondly, the mean size of the capillaries in the tissues of the cretins is increased, and at the same time a greater proportion of the cortical tissue is occupied by large blood vessels than is the case in the normal rat.

The interpretation to be placed on these findings must depend on the validity of the histological preparations as an index of the state of the vascular bed in the living animal. There is no reason to suppose that the density of the vascular network during life should not be satisfactorily represented by the number of fragments of blood vessels present in well-injected specimens, but there is less certainty about the significance of differences in the mean size of these fragments. For example, the enlarged capillaries of the experimental tissues could be regarded as demonstrating not so much a change in the size of the living vessel, as a reduction in the tonus of its wall resulting in passive distension under the pressure of injection. On the other hand, the changes in the proportions of the vascular tree of the hypothyroid rats are consistent with those which would be expected as a result of chronic venous congestion arising from cardiac insufficiency, a disorder frequently associated with cretinism. Yet another possibility is suggested by the observations of Asling, Walker, Simpson, Li & Evans (1952), who concluded that, in rats hypophysectomized in infancy, the brain tends to grow faster than the endocranium. The changes in the

shape of the brain which occur in the cretin (Eayrs & Taylor, 1951) are so similar to those described by Asling *et al.* (1952) as to suggest that early thyroid deficiency is also associated with a disproportion in the growth of brain and skull. This would almost certainly interfere with the venous drainage from the cerebral circulation, and so cause changes in the vascular bed consistent with those observed in the experimental tissues.

Although, on the basis of the present evidence, no choice can be made between the several possible interpretations of the experimental findings, it may be inferred that, from the point of view of the metabolism of the surrounding nervous tissues, the reduced density of the capillary network is a more critical factor than any increase in capillary size which may have occurred in the experimental tissues. One result of a reduction in the number of capillaries is an increase in the mean capillary spacing which must be associated with a corresponding increase in the volume of tissue placed at a nutritional disadvantage by reason of its distance from its blood supply. A second effect must, in the absence of any increase in the size of the vessels, be a reduction in the area of capillary surface available for metabolic exchange. Calculations based on the number and mean size of capillary fragments present in normal and cretinous tissues (Table 1) show that the apparent increase in the size of the capillaries in the tissues of the cretins, although fully restoring the volume of the capillary bed, could restore only about 45 % of the capillary surface area lost as a result of the reduction in numbers.

From these considerations it may be concluded that, whether or not the capillary enlargement seen in the experimental tissues is present during life, the vascular changes which occur in hypothyroidism are consistent with a reduced tissue metabolism. In addition to its influence on vascularity, however, thyroid deficiency is also responsible for alterations in cortical structure, and the fact that simultaneous changes occur in both nervous and vascular tissues raises the problem of the causal inter-relationship between the two effects. Many authors who have studied the factors which regulate the vascularity of the central nervous system (e.g. Aby, 1899; Cobb, 1929; Dunning & Wolff, 1937; Wolff, 1938; Campbell, 1939; Scharrer, 1945), basing their conclusions on the correlation between regional differences in angio- and cyto-architecture, have implied that the density of the capillary network is determined by the structure and metabolism of the nervous tissue which it serves. This suggests that the changes in the vascularity of the brain of the cretin may be secondary to one or more of the concomitant changes which occur in cortical structure, such as an increase in cell density (Eayrs & Taylor, 1951), a reduced myelination (Benda, 1947; Barnett, 1948), or a hypoplasia of both axonal and dendritic components of neuropil (Eayrs, 1953), the reduced number of capillaries being adequate to meet the needs of the modified nervous tissues. On the other hand, the changes in the vascular bed in the cretin are such as to predispose the surrounding tissues to anoxia, a condition known to cause severe and lasting damage to the developing nervous system (Windle, 1944), a fact which suggests that hypothyroidism may influence cortical structure indirectly through its effect on vascularity. The findings of the present experiment do not provide an adequate basis for differentiating between these two possible effects whose dissociation demands the development of more specialized techniques than have so far been applied to the problem.



## SUMMARY

1. The vascularity of the cortex of normal rats and rats made cretinous by giving methyl thiouracil has been measured using the method of injection with carmine-gelatin.

2. There were fewer fragments of capillaries per square millimetre in the cerebral cortex of cretinous rats than in that of the controls. The number of blood vessels which exceeded  $5\mu$  in diameter was, however, greater in the experimental tissues.

3. The overall proportion of cortical tissue occupied by blood vessels was unaffected by thyroidectomy, but vessels exceeding  $5\mu$  in diameter occupied significantly more space in the brains of the cretins than in the normal, and the capillaries rather less.

4. The mean size of the capillary fragments in the tissues of cretinous rats was significantly larger than in those of the controls.

5. There were more capillaries in the supra-granular and granular layers of the cortex than in the infra-granular layers, and these vessels occupied relatively more space. Vessels exceeding  $5\mu$  in diameter also occupied a greater proportion of the more superficial cortical laminae than of the deeper layers, but their total number was similar in both locations.

6. Thyroidectomy had the effect of accentuating certain inter-laminar differences, particularly those involving the number of large vessels in layers 3-5, and in the mean size of vessels in the supra- and infra-granular layers.

7. The proportion of tissue occupied by blood vessels was greater on the lateral aspect of the cortex than on the medial. The mean size and density of capillaries, however, was similar in both regions. These differences were unaffected by the experimental treatment.

8. The significance of these findings is discussed. It is suggested that, from the point of view of tissue metabolism, the reduced number of capillaries in the cretin is of greater importance than any increase in their mean size. There is insufficient evidence, however, to show whether this reduction is the cause or consequence of accompanying changes in cerebral architecture.

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# THE DISTRIBUTION OF RADIOACTIVE SULPHUR ( $^{35}\text{S}$ ) IN THE FIBROUS TISSUES, CARTILAGES AND BONES OF THE RAT FOLLOWING ITS ADMINISTRATION IN THE FORM OF INORGANIC SULPHATE

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Sulphur-containing mucopolysaccharides are widespread in the body and occur particularly in the matrix of fibrous tissues, in cartilage and in the intestinal tract. Though they are generally considered important constituents of the connective tissues, little is known of their precise function or metabolism. Sulphur enters the body mainly in the sulphur-containing amino-acids, methionine and cystine. Smaller amounts enter as inorganic sulphate and in other inorganic forms. Oxidation of the sulphur-containing amino-acids in the body results in the production of inorganic sulphate, some of which presumably participates in the formation of sulphur-containing mucopolysaccharides. By far the major part of the sulphate produced by oxidation within the body is quickly excreted and this is true also for inorganic sulphate administered to an animal.

In the present series of experiments the distribution of radioactive sulphur ( $^{35}\text{S}$ ) in rats after its intraperitoneal injection in the form of sodium sulphate has been studied using radioautography supplemented by counting procedures. The present paper is mainly concerned with the description of its distribution in connective tissues.

## MATERIALS AND METHODS

Male rats were injected intraperitoneally with a solution of  $^{35}\text{S}$ -labelled sodium sulphate, each animal receiving approximately  $2\mu\text{c.}$  of  $^{35}\text{S}$  per gram of body weight. The animals were killed with chloroform after periods varying from half an hour to 10 days, and tissues were removed for radioautography and counting purposes. The precise details are set out in Table 1.

For radioautography in the earlier experiments the tissues were fixed either in 10% formalin or in absolute ethyl alcohol. The formalin-fixed material was washed in water, passed through the alcohols to absolute alcohol, embedded in paraffin wax, sectioned and mounted in the usual way. The paraffin was removed with xylol and the slides passed down the alcohols to water. Then, in a dark-room, they were covered with a strip of Kodak radioautography film, and dried. Those fixed in absolute alcohol were passed through xylol, cleared in benzene, embedded in wax and sectioned. One series of sections was thereafter treated like the formalin-fixed tissues, whilst another series was mounted without the aid of any fluid, and the paraffin wax was not removed prior to exposure, each slide being clamped in the dark-room to another slide bearing the dried film strip mounted in the usual manner (contact radioautography). Known volumes of the fixatives used in these early



experiments, as well as any water used in mounting, were evaporated to dryness and the radioactivity of any residue estimated. Kidney tissue was used in these preliminary experiments; formalin fixation and mounting with water resulted in appreciable loss of radioactive material. Consequently most of the radioautographs were done on material fixed in absolute alcohol, mounted without the use of water and clamped to another slide bearing the stripping film. As will be seen in later experiments the loss of radioactive material from cartilage as a result of washing with water was not nearly so marked. Bony tissues were not decalcified and the paraffin wax was not removed until the exposure had been completed. In all cases two sections were mounted on each slide, the one cut at  $10\mu$  and the other at  $50\mu$  thickness. During exposure the slides were stored in a light-proof box in the refrigerator at  $0^\circ\text{C}$ .

After the appropriate exposure the pairs of slides were separated, the film strip developed, fixed and dried and the sections passed through xylol to remove the

Table 1. *Table of individual experiments*

Weight of rat (g.)	Age of rat at beginning of experiment	Duration of experiment	Vol. injected (ml.)	S injected (mg.)	S per g. rat ( $\mu\text{g.}$ )	$^{35}\text{S}$ ( $\mu\text{c.}$ )	$^{35}\text{S}$ per g. ( $\mu\text{c.}$ )	Weight of rat at end of exp. (g.)
6.3	2 days	4 hr.	0.25	0.050	7.9	11.6	1.84	—
9.7	2 days	4 hr.	0.37	0.074	7.6	17.1	1.76	—
30.4	3 weeks	0.5 hr.	0.25	0.25	8.2	59.6	1.96	—
30.5	3 weeks	4 hr.	0.25	0.25	8.2	60.7	1.99	—
30.0	3 weeks	4 hr.	0.36	0.25	8.3	59.4	1.98	—
26.0	3 weeks	4 hr.	0.25	0.25	9.6	74.0	2.85	—
30.0	3 weeks	4 days	0.25	0.25	8.3	74.0	2.47	31.4
28.0	3 weeks	6 days	0.25	0.25	8.9	74.0	2.64	31.0
30.5	3 weeks	10 days	0.25	0.25	8.2	60.0	1.97	34.9
190.0	Mature rat	4 hr.	0.5	1.67	8.6	400.0	2.05	—

wax and then stained with haematoxylin and eosin and mounted in balsam in the usual way.

The duration of exposure was determined in a preliminary experiment on 25% gelatin mixtures containing 1 mg. inorganic sulphate-S per ml. and 25, 2.5, 0.25 and  $0.025\mu\text{c. }^{35}\text{S}$  per ml. From these, cylindrical blocks were removed with cork borers, and  $100\mu$  sections were cut on the freezing microtome. These were placed in contact with the film and stored in the dark, in a refrigerator, for 1–12 weeks. Optimum registration was obtained in the 2nd and 3rd weeks, and consequently, with all tissues, two sets of slides were mounted, the one being exposed for 2 weeks, the other for 3 weeks.

In the earlier experiments control rats of the same age (litter-mates) were injected with similar doses of non-radioactive sodium sulphate and the tissues treated in parallel with those from experiments using  $^{35}\text{S}$ .

The techniques used for the measurement of radioactivity by means of counting equipment were based on those described by Henriques, Kistiakowsky, Margnetti & Schneider (1946) and Young, Edson & McCarter (1949). Weighed portions of tissue in amounts up to about 0.25 g. were oxidized with nitric acid by the Carius procedure. At the end of the oxidation the sulphuric acid present was precipitated as benzidine sulphate which was then filtered. The radioactivity of the precipitate

was measured with counting equipment of the usual type employing a Geiger-Müller tube with a thin end-window.

The buffer with pH 10 used for washing the tissues and sections in experiments to be described later was a 0.2N-KH<sub>2</sub>PO<sub>4</sub>-NaOH buffer prepared as described by Britton (1942), and it was checked by means of a pH meter.

## RESULTS

### *Effects of fixatives and use of water in mounting*

When formalin was employed as a fixative and water for washing or mounting sections, the material lost radioactivity and the formalin or water became radioactive. When absolute alcohol was the fixative it did not acquire any detectable radioactivity, but even after alcohol fixation mounting the section with water still caused loss of radioactivity.

From three successive 10  $\mu$  sections of the same block of tissue fixed in absolute alcohol, one section mounted with water gave a direct count of 5 per minute, another mounted without water gave one of 26 per minute with the paraffin wax *in situ*, whilst a third mounted dry and deparaffinized gave a count of 29 per minute. The effect of the thickness of tissue sections was also tested and the results obtained are shown in Table 2.

Table 2. *Direct counts from successive sections of kidney of increasing thickness*

Section no.	Thickness of section ( $\mu$ )	Counts per min.
1	10	68
2	20	94
3	30	124
4	40	127
5	50	139
6	100	172

In subsequent work, sections of 50 and 10  $\mu$  were taken, the former being easier to manipulate and yielding intense radioautographs giving the gross distribution of radioactive material, whilst the latter, though giving less intense images, permitted a more detailed histological examination.

### *Distribution of <sup>35</sup>S*

Both in the 2-day-old and in the 3-week-old (approximately 30 g.) rats the radioactive sulphur was distributed widely through the tissues 4 hr. after the injection. It could not be detected, however, in the epidermis, hair, claws and lens of the eye. Blood, muscle, bone marrow, liver and kidney showed a diffuse and more or less even distribution of radioactive sulphur. Greater radioactivity occurred in the fibrous tissues; intermuscular septa were clearly outlined on the photographic film, as were joint capsules, tendons, ligaments and the sclera of the eye. The denser the connective tissue, the more intense the radioautograph and presumably the greater their content of radioactive sulphur. Thus the images of the intermuscular septa stood out in contrast to the muscles whilst the capsules and ligaments of joints and the sclera of the eye and substantia propria corneae produced even denser radioautographs. The dermal layer of the skin was clearly shown, as were

the iris of the eye and the ciliary body, giving images approaching in intensity to those of the intermuscular septa. These structures in the eye stood in marked contrast to the retina which gave a poor radioautograph. Still more intense radioautographs were obtained from the trabeculae of bone, which stood out in contrast to the bone marrow. The superficial layer of the periosteum, perichondrium, and the main parts of tendons yielded images of an intensity equal to that of joint capsules and ligaments. Near their attachment to the bone, however, the tendons showed greater radioactivity than elsewhere (Fig. 1A, B). The loose connective tissue of synovial membranes yielded a fainter image than the ligamentous and capsular structures.

The most intense radioautographs were obtained from cartilage. All cartilages showed considerable radioactivity, least in the menisci of joints which gave images of an intensity comparable to those of capsular ligaments, and greatest in epiphyseal

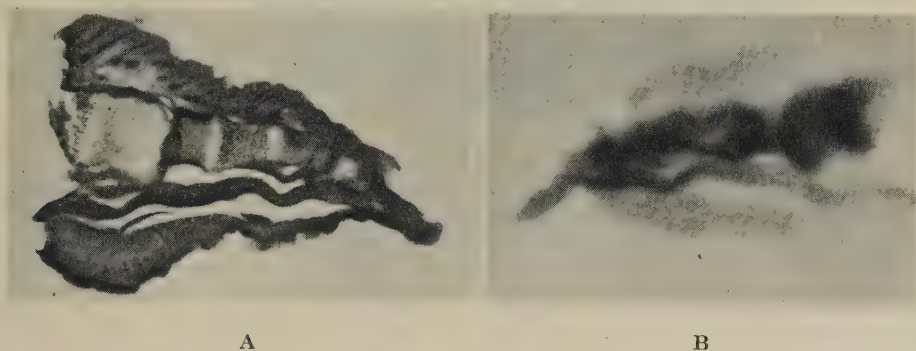


Fig. 1A, B. Sagittal section of a digit with the corresponding contact radioautograph from a 2-day-old rat (no. 2) injected 4 hr. previously with radioactive sodium sulphate. Note the intensity of the radioautograph of the cartilaginous epiphyses and of the tendon. Haematoxylin and eosin.  $\times 18$ .

cartilages (Fig. 2A, B). In more or less homogeneous masses of cartilage like the bulk of the nasal septum, the alae of the nose, the cartilage of the pinna (Fig. 3A, B), and the cartilaginous epiphyses, there was a more or less even distribution of radioactive matter, which gave slightly more intense radioautographs than the perichondrium. In epiphyseal cartilage the greatest concentration of radioactive material occurred in the zone of the cartilage cell columns. At the edges of the epiphyseal disc this dense zone became continuous with another and possibly slightly less dense zone corresponding to the deeper, osteogenic layer of the periosteum. There was a diminution in the intensity of the radioautograph on passing from the zone of the cartilage cell columns either towards the calcified zone adjoining the diaphysis or towards the epiphysis. All growing cartilages showed this marked accumulation of radioactive sulphur in the corresponding zone of cell columns. This was well seen in the upper part of the nasal septum, just in advance of the ossification extending into it from the ethmoid, in the epiphyseal discs of all long bones and in the growth cartilage at the base of the skull between the basisphenoid and basiocciput and between the basisphenoid and presphenoid. In



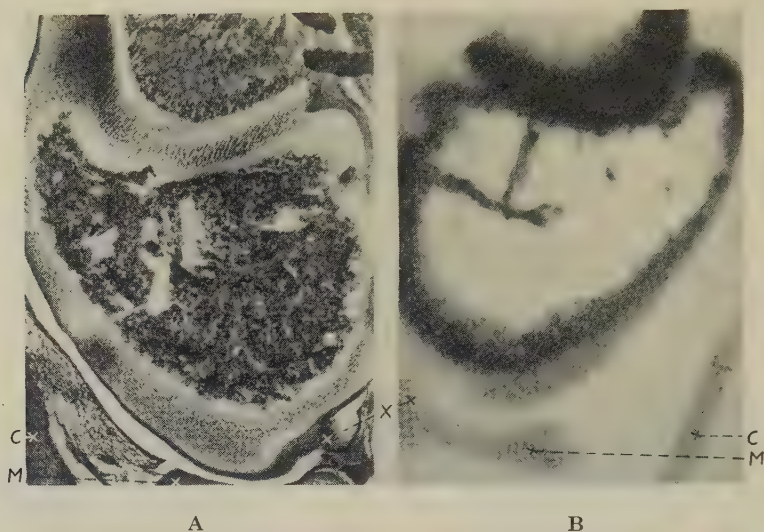


Fig. 2 A, B. Sagittal section of the lower end of the femur and knee joint with the corresponding contact radioautograph from a 2-day-old rat (no. 2) injected 4 hr. previously with radioactive sodium sulphate. C, capsule; M, meniscus; X, cruciate ligament. Haematoxylin and eosin.  $\times 18$ .

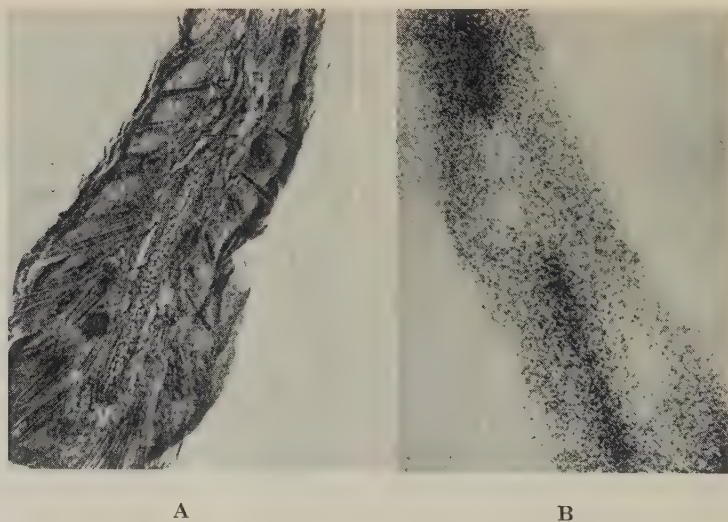


Fig. 3 A, B. Section of pinna with corresponding contact radioautograph from a 3-week-old rat (no. 6) injected 4 hr. previously with radioactive sodium sulphate. Note the density of the radioautograph of the elastic cartilage. Haematoxylin and eosin.  $\times 67$ .

articular cartilage there was a corresponding localized accumulation of radioactive material just outside the zone of cell division or mitotic annulus (Harris, 1933). In the adult rat the radioautograph was most intense again in the zone of the cell columns of the epiphyseal cartilage, though possibly less so than in the corresponding zone of the young animal. In the rat the epiphyseal discs remain unossified into adult life.

Thus 4 hr. after the injection as judged by the intensity of the radioautograph there were localized accumulations of radioactive sulphur in the connective tissues, more marked where these were dense, and still greater concentrations in cartilage, particularly in the zone of the cartilage cell columns of growing animals.

In the only rat killed within half an hour of the injection (Exp. 3) the differential distribution of radioactive sulphur was similar to that in the 3-week-old and mature rats killed 4 hr. after injection, though the intensity of the radioautographs and the estimates made with the counter were lower. The latter are given in Table 3.

Table 3. *Measurements of the radioactivity of some skeletal tissue from a 3-week-old rat (no. 3), killed half an hour after injection*

Tissue	Radioactivity in counts per mg. wet weight per min.
Nasal septum	52
Articular and epiphyseal cartilages, upper tibia	86
Articular and epiphyseal cartilages, upper end of femur	78

Table 4. *Radioactivity of whole carcasses*

Animal no.	Duration of exp.	Weight at commencement of exp. (g.)	Weight at end of exp. (g.)	Radioactivity in counts per mg. tissue per min.
6	4 hr.	26.0	—	70
7	4 days	30.0	31.4	2
8	6 days	28.0	31.0	2

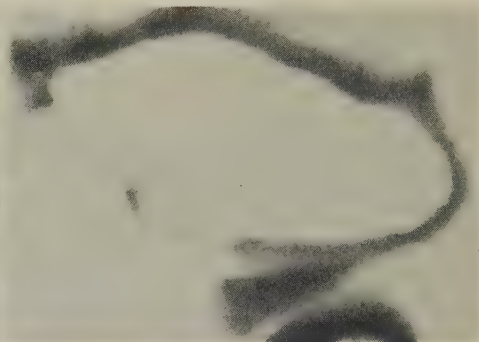
As to the radioactivity of the tissues at varying intervals after the injection, three rats (nos. 6–8) were injected with comparable doses of radioactive material and killed at 4 hr., 4 and 6 days respectively. Estimates of the amount of radioactive material retained at the end of the experiment gave the results shown in Table 4. A fourth animal (Exp. 9) was later injected and kept for 10 days after the injection, but no measurements of radioactivity were made.

The animals kept for 4, 6 and 10 days all gained in weight, the last-mentioned weighed 30.5 g. at the commencement of the experiment and gained 4.4 g. during its course. The radioautographs at 4 hr. have already been described. At 4 and 6 days after the injection no image was produced by muscle, blood, the loose or dense fibrous tissues, the sclera or cornea of the eye, the capsules, ligaments of joints or tendons. All cartilages, except that of the pinna, yielded radioautographs comparable in intensity and distribution of radioactive material to those obtained 4 hr. after injection (Figs. 4A, B; 5A, B). The radioautograph of the fibrocartilage of the pinna was much less intense. This diminution was still more marked at 6 days.

Furthermore, 6 days after the injection the decrease in intensity of the radioautograph produced by the cartilages at the upper end of the humerus was much more marked than in the case of the lower end of the femur or the upper end of the tibia, particularly in the zone of the cartilage cell columns of the epiphyseal disc. By 10 days the diminution of radioactivity in all cartilages was much more marked

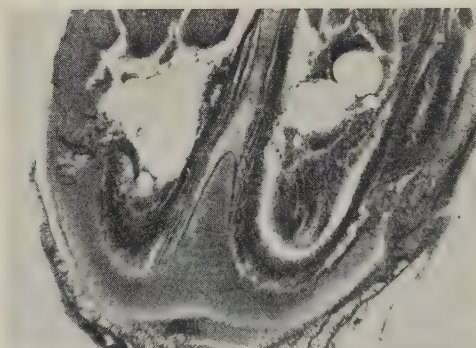


A

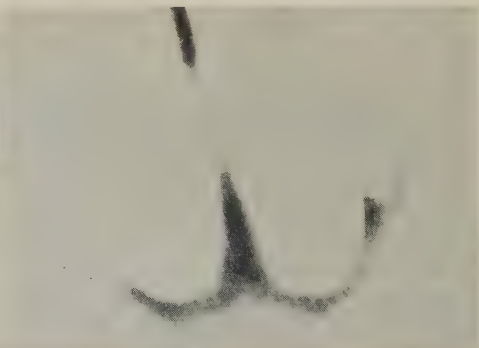


B

Fig. 4A, B. Section of lower end of femur and knee joint with the corresponding contact radioautograph from a 3-week-old rat (no. 8) 6 days after the injection of radioactive sodium sulphate. The epiphyseal, articular and intra-articular cartilage have produced well-marked radioautographs. Haematoxylin and eosin.  $\times 18$ .



A



B

Fig. 5A, B. Section through the tip of the nose with the corresponding contact radioautograph from a 3-week-old rat (no. 7) 4 days after the injection of radioactive sodium sulphate. The septal and alar cartilages have produced well-marked radioautographs. Haematoxylin and eosin.  $\times 18$ .

and the zone of the cell columns could not be identified any longer by the intensity of its radioautograph. Counts directly from the surfaces of the blocks, however, showed that small amounts of radioactivity remained in the epiphyses or adjoining cartilages.

In attempts to remove the radioactive material from the cartilages by washing in distilled water and in buffer solution at pH 10, both fixed and unfixed tissues were taken. After washing sections of cartilage, either in the fresh state or subse-



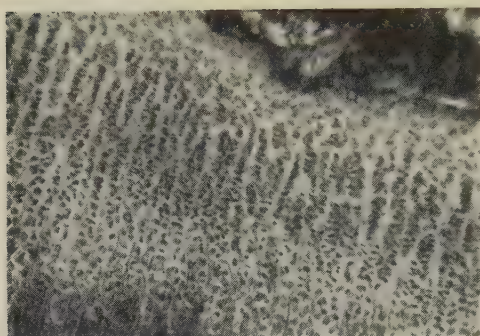
quent to fixation, there was a diminution in the intensity of all radioautographs. After washing, either in distilled water or buffer solution, the radioactive material was no longer uniformly distributed throughout the cartilage, being retained mainly in the pericellular regions of the matrix, so that each cartilage cell was surrounded by a halo of reduced silver on the film. (Compare Figs. 6A, B, 7 and 8.) The matrix furthest removed from the cells still contained some, but much less, radioactive sulphur. The zone of the cell columns continued to give the most intense image. The radioautograph was less intense after the use of buffer than after distilled water, suggesting that the removal of radioactive material was greater in the former case. That some movement of radioactive material might have occurred, which led to an intensification of the radioactivity of the pericellular matrix, cannot be excluded. Both these methods failed to remove even the major portion of radioactive material from any part of the cartilage, either articular, epiphyseal, nasal or auricular. It would seem that in all regions of these cartilages, and more particularly in the cell column zone, much of the radioactive sulphur was fixed in the tissues within 4 hr. and not merely held as inorganic sodium sulphate.

#### DISCUSSION

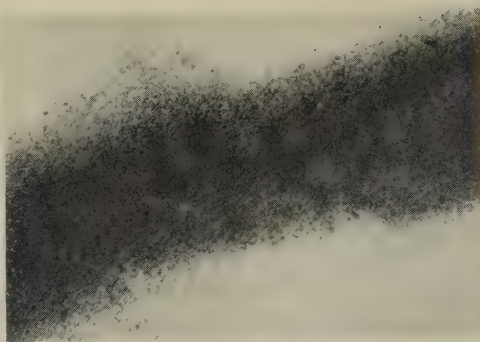
These experiments resemble those of Dziewiatkowski (1952) who confined his observations to the 7-day suckling rat and described the distribution only in the humerus, femur and tibia. As fixatives he used 3.7% formaldehyde with a pH of 3.8–3.9, and 3.7% formaldehyde saturated with barium hydroxide. It is not clear whether he used water in covering the section with the film, nor is there any indication that the fluids used in these procedures were subsequently tested for radioactive materials. Dziewiatkowski (1952) reported that the maximum concentration of  $^{35}\text{S}$  occurred at the junction of the epiphysis and diaphysis.

It is clear from the present experiments that the uptake of  $^{35}\text{S}$  in appreciable quantities occurs in all cartilages, whether fibrous or hyaline, though more markedly in the latter. The uptake is less in white fibro-cartilage such as the menisci than in elastic fibro-cartilage like that of the pinna. Furthermore, in epiphyseal cartilage the maximum concentration occurs in the zone of the cell columns.

As to the distribution of the  $^{35}\text{S}$  in the tissues generally, it seems to occur in greatest concentration in those situations known to be rich in sulphate-containing mucopolysaccharides. That some of it occurs here in the form of inorganic sulphate seems likely from the fact that a proportion is removed even by distilled water. The remainder, however, seems to be fixed in the tissues in some other form—possibly as chondroitin-sulphate or by substitution in some other compound. In the former case one must assume the utilization of the inorganic sulphate in the synthesis of chondroitin sulphate. Dziewiatkowski (1951) has shown radioactive sulphur to exist in the chondroitin sulphate of knee-joint cartilage following the administration of radioactive sodium sulphate. Failure to remove the whole of the radioactive material from cartilage with buffer solution at pH 10 suggests that it is bound in some form other than simple chondroitin sulphate or that the acid mucopolysaccharide is bound to the protein of the cartilage in a form which is not readily dissociable. The occurrence of the radioactive sulphur in maximal concentration in the zone of the cell column (Streeter's phase 4 in particular), where the



A



B

Fig. 6A, B. Section of the lower femoral epiphyseal cartilage with the corresponding contact radioautograph from a 3-week-old rat (no. 4) 4 hr. after the injection of radioactive sodium sulphate. The tissue was fixed in absolute alcohol, sectioned and mounted without the use of water. For comparison with Fig. 8. Haematoxylin and eosin.  $\times 67$ .

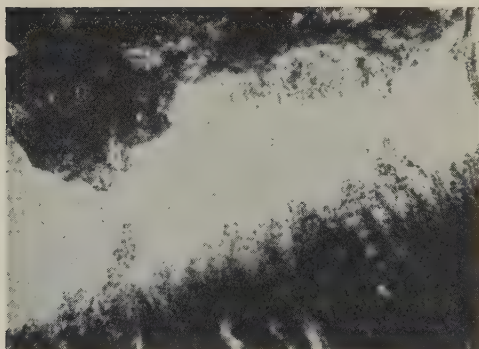


Fig. 7.

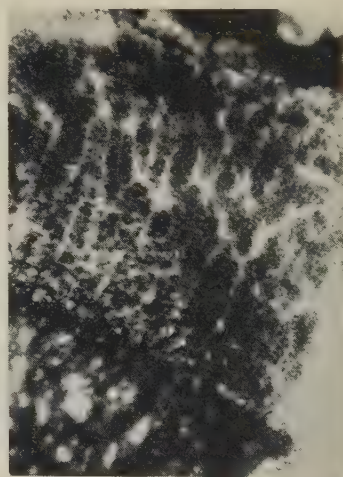


Fig. 8.

Fig. 7. Section through the lower epiphyseal cartilage of the femur of an uninjected 3-week-old rat. The tissue was not decalcified or stained but the section was covered with radioautographic film, placed in the refrigerator for 3 weeks, and the film was subsequently developed and fixed. It served as one of the controls for the sections shown in Fig. 8, with which it should be compared. The light area is the epiphyseal cartilage.  $\times 67$ .

Fig. 8. Section of the lower epiphyseal cartilage of the femur from a 3-week-old rat (no. 4) injected 4 hr. before killing with radioactive sodium sulphate. The tissue was not decalcified but the section was washed in repeated changes of a buffer solution of pH 10 for 4 hr. before being covered with a radioautographic film and placed in the refrigerator for 3 weeks. The film was then developed and fixed but the section was not stained.  $\times 67$ .

proportion of cartilaginous matrix to cells is also maximal, suggests that it has been incorporated into the matrix by synthesis. Furthermore, there is a marked diminution in concentration as the calcified cartilage zone and bone is reached.

As regards the change in concentration of isotope in the epiphyseal cartilages and other tissues with time, Dziewiatkowski, Benesch & Benesch (1949) attributed this mostly to a dilution effect due to the newly formed cartilage in growing animals. This would hardly account for its disappearance from the fibrous tissues in these experiments, nor is it likely to explain its disappearance from the cartilages of the pinna and nasal septum. A part of the radioactive sulphur deposited in the zone of the cell columns might well disappear as a result of growth processes, but elsewhere the disappearance must occur largely as a result of the continual replacement of the tissue constituents which is known to occur.

### CONCLUSIONS

1. After intraperitoneal injection of  $^{35}\text{S}$ -labelled sodium sulphate, the radioactive sulphur is found in highest concentration in the zone of the cell columns of epiphyseal cartilage. It occurs, however, in quantity in all cartilages, whether fibrous or hyaline, and in lesser concentration in the fibrous tissues.

2. Radioactive sulphur disappears from the fibrous tissues and from most cartilages except from the cell column zones by the 10th day after injection.

3. Four hours after injection some of the sulphur in these situations is in the form of inorganic sulphate, the remainder exists in a form insoluble either in distilled water or in buffer solution at pH 10. The latter occurs principally in the pericellular zone and particularly in the region of the cartilage cell columns. The evidence suggests that the inorganic sulphate has been incorporated in a component of the cartilaginous matrix.

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# THE EFFECT OF PREGANGLIONIC SECTION ON THE NEURONS OF THE SUPERIOR CERVICAL GANGLION IN RABBITS

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## INTRODUCTION

There is evidence that at least in some cases if the afferent connexions of a neuron are allowed to degenerate the perikaryon undergoes morphological changes. These changes, known collectively as transneuronal degeneration, are especially marked in the cells of the lateral geniculate body following section of the optic nerve (Minkowski, 1920; Clark, 1932; Clark & Penman, 1934). In this situation the process is rapid and more complete in primates and slower and less severe in other mammals such as the rabbit and cat (Minkowski, 1920, 1922; Cook, Walker & Barr, 1951).

Transneuronal degeneration has been described elsewhere in the central nervous system. Warrington (1898, 1899) found chromatolysis in the spinal cord of the cat and the monkey following dorsal root section or transection of the cord. Barron (1933) described chromatolysis in anterior horn cells after section of the pyramidal tract. Foerster, Gagel & Sheehan (1933) have described transneuronal effects in the spinal cord following posterior root section, and Foerster & Gagel (1934) found degenerative changes in the cuneate nucleus after section of the posterior columns.

Other writers (Schimert, 1939; Hare & Hinsey, 1940) have failed to confirm the occurrence of transneuronal degeneration in spinal cord neurons. Cook *et al.* (1951), in a study involving section of cortico-spinal tracts or dorsal root fibres, found that '...no cell changes were observed in experimental animals which could not be found in control animals or explained by factors other than partial isolation'.

From these conflicting results it would appear either that the transneuronal effect is not so pronounced in the case of spinal cord neurons, or that the effect depends on total isolation of the neuron.

It is possible that neurons in different parts of the nervous system react to a varying extent to deafferentation. Clark (1943) considers that the severe reaction found in the cells of the lateral geniculate body after optic nerve section is '...indicative of the extreme specificity of function of the cells in this nucleus'.

Complete isolation of neurons is also possible in the autonomic nervous system, e.g. in the superior cervical ganglion by cutting the preganglionic trunk. The reaction of the cells of this ganglion is not so severe as that of the cells of the lateral geniculate body. The changes which have been described following preganglionic section are shrinkage of the neuron with some crenation of its margin and a tendency of the Nissl substance to become aggregated at the periphery of the cell (Sternschein, 1920; de Castro, 1923). However, Gibson (1940) failed to find any changes in the ganglion cell bodies of the superior cervical ganglion following preganglionic section.

The present study is a reinvestigation of the transneuronal effect in the superior cervical ganglion. An attempt has been made to estimate any change in cell size by quantitative methods.

#### MATERIAL AND METHODS

Adult rabbits of various breeds and both sexes were used. Anaesthesia was begun with pento-barbital sodium (Nembutal), the dose given being 30 mg./kg. body weight; this was supplemented where necessary by open ether. Using full aseptic precautions the cervical sympathetic trunk was exposed at the level of the cricoid cartilage and then stimulated by means of a faradic current. The resulting dilation of the pupil was taken as confirmation of the identity of the isolated nerve.

The trunk was then divided as low down as possible and the cut ends sutured into muscle so as to prevent regeneration. The trunk of the opposite side was exposed and stimulated but not divided.

Survival times were allowed up to 150 days, the animals being biopsied under Nembutal-ether anaesthesia. The superior cervical ganglia and the part of the cervical sympathetic trunks immediately caudal to them were removed. The ganglia were allowed to adhere to small cards and then fixed in Bouin's fluid (picric acid 75 ml.; 40 % formaldehyde 25 ml.; glacial acetic acid 5 ml.), while the nerves, stretched on cards in which a window had been cut, were put into Bodian's fixative (40 % formaldehyde 15 ml.; glacial acetic acid 5 ml.; absolute alcohol 80 ml.).

After embedding in paraffin the ganglia were cut longitudinally at  $5\mu$  thickness and the majority of the mounted sections stained with Heidenhain's azo-carmin and aniline blue. Some sections, however, were stained with buffered thionin or Borrel's blue in order to observe changes in the Nissl substance. In this respect it may be noted that the azo-carmin stain, in autonomic ganglia also gives a good picture of the Nissl bodies.

As the ganglion of the unoperated side was used as a control, the operated and unoperated ganglia from a particular animal were put through the staining and photographic processes together, and as far as possible under identical conditions.

The problem of making a quantitative estimate of cell size in the superior cervical ganglion is rendered difficult by the fact that the density of the connective tissue stroma does not permit the use of the method of optical section for measuring the diameter of the neuron.

The following procedure was therefore used. Photographs were taken at 750 diameters of random fields. In each field the eight largest cells showing at least one nucleus and nucleolus were traced on to good-quality tracing paper. The resulting outlines were cut out and weighed using a micro-balance, the weights being then converted to square micra. One hundred cell areas were measured for each ganglion and the mean of these results calculated. The figure from the operated side was then compared statistically with that of the control ganglion.

The method of tracing, cutting out, and weighing for the estimation of small irregular areas has been previously discussed by Scammon & Scott (1927) and Mainland (1929). The latter finds that celluloid of uniform thickness gave the greatest accuracy, but this is laborious to work with.

The use of tracing paper has the advantage that the cell outline is easily traced and then cut out with scissors. As it was thought possible that the thickness of the paper would not be sufficiently uniform to give the required degree of accuracy, a survey was carried out taking samples from the beginning, the middle and the end

of a roll of paper. Standard areas were punched out and on weighing these it was found that the variation was in fact very small (Table 1). The accuracy of the tracing and cutting out was tested by making a number of estimates of the same cell. These were found to be in close agreement (Table 2).

Table 1. *Weight in grams of standard discs 1 cm. in diameter, punched from a roll of tracing paper 30 in. wide and containing 60 ft. of paper. Samples of twelve discs each were taken at the beginning and end of the roll and at 20 ft. intervals between the two*

	0 ft.	20 ft.	40 ft.	60 ft.
	0.0036	0.0040	0.0038	0.0040
	0.0040	0.0038	0.0040	0.0041
	0.0042	0.0039	0.0039	0.0040
	0.0040	0.0040	0.0038	0.0040
	0.0038	0.0039	0.0040	0.0042
	0.0038	0.0040	0.0040	0.0040
	0.0038	0.0039	0.0040	0.0040
	0.0040	0.0040	0.0040	0.0039
	0.0040	0.0040	0.0039	0.0040
	0.0040	0.0039	0.0039	0.0040
	0.0039	0.0038	0.0040	0.0040
	0.0040	0.0040	0.0040	0.0042
Mean				
weight (g.)	0.0039	0.0039	0.0039	0.0040

Table 2. *Weight in grams of the outlines of two neurons cut out of tracing paper. Fifteen outlines were weighed from each neuron*

	1st neuron	2nd neuron
	0.0183	0.0217
	0.0176	0.0222
	0.0174	0.0220
	0.0174	0.0219
	0.0174	0.0220
	0.0178	0.0220
	0.0182	0.0212
	0.0176	0.0218
	0.0176	0.0222
	0.0178	0.0228
	0.0176	0.0226
	0.0180	0.0226
	0.0176	0.0230
	0.0176	0.0220
	0.0182	0.0223
Mean	0.0177	0.0222
S.D. of the mean	<0.0001	<0.0001

## RESULTS

The changes which have been described in the neurons of the superior cervical ganglion following preganglionic section are mild chromatolysis, crenation of the cell margin, aggregation of Nissl bodies at the periphery and displacement of the nucleus to the side of the cell (Sternschein, 1920).

In the present investigation chromatolysis was found to be difficult to detect. The Nissl substance in the neurons of the superior cervical ganglion is powdery in appearance, so that evidence such as the fragmentation of coarse granules, characteristic of chromatolysis in spinal motoneurons, is not found. However, the Nissl substance stains less easily after preganglionic section, the cells appearing paler.



This change is first seen at about 40 days after operation and persists at 160 days (Pl. 1, figs. 2, 4 and 6).

The other changes mentioned were not found to be characteristic of the deafferented ganglion. Crenation of the cell margin, peripheral aggregation of Nissl bodies and eccentric position of the nucleus were all noted in normal ganglia (Pl. 1, figs. 3, 5). A noticeable feature of the ganglion of the operated side is the greater amount of stroma (Pl. 2, figs. 2, 4 and 6), which shows an increase in the number of non-neuronal nuclei, as might be expected from the increase in Schwann cell population in the presence of degenerating preganglionic myelinated axons. Joseph (1950) has shown that there is an increase in the nuclear count of nerves containing small myelinated fibres during degeneration, but not in the case of nerves containing non-myelinated axons only.

It is difficult to estimate qualitatively any change in cell size in the operated compared with the normal ganglion. This difficulty is partly due to the fact that few neurons are cut equatorially, and partly to the increase in connective tissue stroma.

On considering the results of the measurement of cell areas (Table 3), it is seen that although the mean cross-sectional area of the neurons of the deafferented side is smaller than that of the control for the 20- and 30-day survival times, the difference only becomes statistically significant at 40 days.

Table 3. *Changes in neuronal area following isolation*

Survival time in days	Mean area of 100 neurons ( $\mu^2$ )		Difference ( $\mu^2$ )	Difference (%)	Significant ? ( <i>t</i> test)
	Normal ganglion	Deafferented ganglion			
20	750	720	30	4.0	No
30	827	790	37	4.4	No
40	713	649	64	8.9	Yes
80	817	734	83	10.1	Yes
100	839	694	145	17.2	Yes
160	682	559	123	18.0	Yes

This reduction in size progresses from 8.9 % at 40 days to 17.2 % at 100 days and is still of about the same order (18 %) at 160 days.

## DISCUSSION

The time course and degree of the neuronal shrinkage observed in these experiments is of about the same order as that described by Cook *et al.* (1951) in the neurons of the lateral geniculate body of the cat following eye enucleation. They found that reduction in cell size appeared at 63 days and reached a level of 25 % of the cross-sectional area of the cell body, where it remained constant up to a survival period of 10 months. The interpretation of the morphological changes which occur in isolated neurons is difficult and involves consideration of the cytochemistry of the Nissl bodies as well as alterations in the neuron and its environment.

The significance of the changes which occur in chromatolysis of neurons is not clear. It has been shown by Caspersson (1940) and Landström, Caspersson & Wohlfart (1941) that the Nissl substance consists essentially of nucleoproteins, the

nucleotide components being possibly of the ribose type. Cook *et al.* (1951) found that the reduction in neuronal volume in the deafferented neurons of the lateral geniculate body was accompanied by depletion of the Nissl substance and diminution in size of the nucleus and nucleolus. They suggested that these findings may imply a depression of nucleic acid and nucleoprotein metabolism, since the nucleus and especially the nucleolus is intimately concerned with the synthesis of Nissl bodies (see Hydén, 1943). Furthermore, these authors point out that the inactivity resulting from deafferentation may be partly responsible for this depression, as Hydén has also reported that the metabolism of ribose nucleoprotein is influenced by electrical stimulation of the neuron. On the other hand, Liu, Bailey & Windle (1950), in a critical investigation of the effect of electrical stimulation on neurons, were unable to confirm Hydén's findings.

Gersh & Bodian (1943) investigated the chemistry of chromatolysis in spinal motoneurons following dorsal and ventral root section. They used ultra-violet absorption methods similar to those of the Stockholm school, and found insufficient evidence to determine the nucleus or the nucleolus as the site of replacement of the Nissl bodies. Their work suggests that although the nuclear changes in nucleotide and protein components during chromatolysis may be similar to those taking place in the cytoplasm, they occur to a much smaller extent. As a result of their studies and those of Schoenheimer (1942), these authors consider that 'the enzyme mechanisms disturbed in chromatolysis are present both in the nucleus and the cytoplasm'.

There is a tendency to refer to all changes in the appearance of the Nissl substance of neurons as chromatolysis, with the implication that the physico-chemical mechanism underlying such changes is similar in all cases. Alteration in the Nissl bodies occurs in widely differing circumstances; as a result of disease, the action of toxins, axonal section or isolation of neurons. It seems unlikely that the chemical changes undergone by the Nissl material are the same in all instances. That such changes may not be always of the same kind, even as judged by microscopical observation is seen from the attempt to classify chromatolysis into stages by Campbell & Novick (1946). These authors investigated the spinal cord of cats following section of the sacral roots and observed that the affected neurons varied in two directions from normal. Three groups of neurons showed the progressive disintegration of the Nissl bodies usually described as chromatolysis, whereas in two other groups the Nissl particles were larger and more intensely staining than normal. The time course of the changes occurring in transneuronal degeneration is different from that of chromatolysis resulting from axonal section. The earliest onset of transneuronal degeneration is seen in the lateral geniculate body of primates 7 days after isolation (Clark, 1943), whereas the chromatolytic change following axon section is apparent 43 hr. following operation (Gersh & Bodian, 1943).

Chromatolysis is associated with changes in cell size. Gersh & Bodian (1943) noted in their experiments that the chromatolytic neurons appeared more spherical in outline, and that they had increased in volume.

In the case of transneuronal degeneration, as seen in the lateral geniculate body or the superior cervical ganglion, the neurons undergo a progressive reduction in size, no phase having been recorded in which the neuronal volume is increased,

except that swelling of neurons which have undergone deafferentation has been described in the sensory nucleus of the fifth nerve about 100 days after alcohol injection of the Gasserian ganglion (Penman & Smith, 1950).

The reaction of the neurons of the superior cervical ganglion to deafferentation is not as severe as that which occurs in the lateral geniculate body, either in the amount of neuronal shrinkage, or in the degree of severity of the chromatolytic reaction. It may be that sympathetic ganglion cells are less sensitive to changes in their environment than the neurons of the lateral geniculate body. Murray & Stout (1947) observed migration and division of sympathetic ganglion cells from adult animals when cultured *in vitro*. This does not appear to have been demonstrated in the case of neurons from the central nervous system and may indicate greater powers of autonomy in the case of the ganglion cells.

Little is known about the functional condition of neurons which have been subjected to deafferentation. No investigation of the physiological state of the neurons of the lateral geniculate body following isolation appears to have been attempted.

According to Gibson (1940) the soma potentials of isolated neurons of the superior cervical ganglion appear normal. This was judged by firing antidromic impulses down the postganglionic trunk after preganglionic section. However, antidromic invasion of the neuronal soma does not occur physiologically, and the interpretation of such results is difficult (Eccles, 1950).

Also in the case of the superior cervical ganglion, Cannon & Rosenblueth (1936) observed that as indicated by contractions of the nictitating membrane, its neurons were more sensitive to circulating acetylcholine after deafferentation.

In the peripheral autonomic nervous system it seems that denervated structures generally show an increased irritability to circulating chemical agents, as stated in Cannon's 'Law of Denervation' (1939), and the 'Law' has since been extended to the central nervous system, as seen from the work of Drake & Stravsky (1948). So far, this increased sensitivity of denervated structures has not been explained.

Complete deafferentation of neurons is practicable only in certain situations such as the lateral geniculate body or the superior cervical ganglion. Such complete isolation is always followed by the morphological changes known as transneuronal degeneration; there is no definite evidence of change in the case of partially isolated neurons. It is likely that changes in the immediate environment of the neuron, such as the disintegration of afferent axons and the disappearance of synaptic terminals, together with the reaction of the connective tissue, play an important part in establishing transneuronal degeneration. The absence of normal stimulation may affect the neuronal metabolism, but there is no certain evidence of this.

#### SUMMARY

1. A qualitative and quantitative study has been made of the changes which occur in the neurons of the superior cervical sympathetic ganglion of the rabbit following preganglionic section.

2. The changes observed were less severe than those which have been described for some neurons of the central nervous system during transneuronal degeneration. In the isolated ganglion the Nissl substance did not stain as strongly as in the



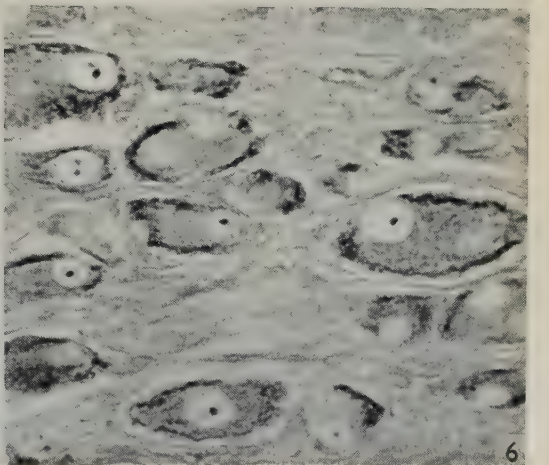
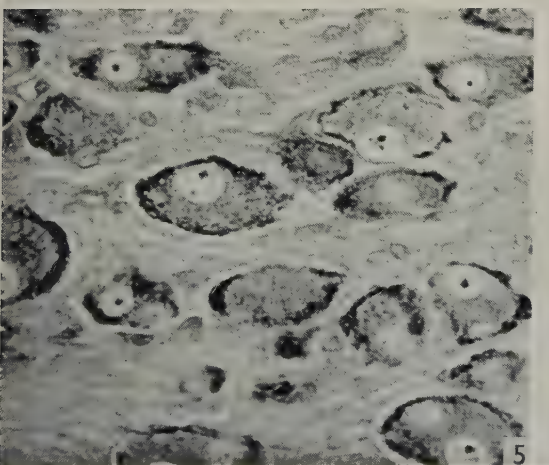
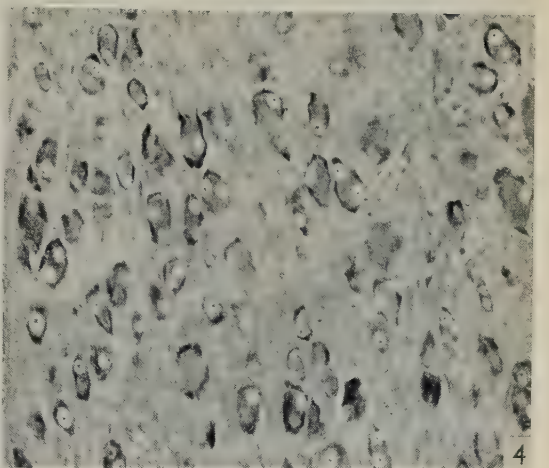
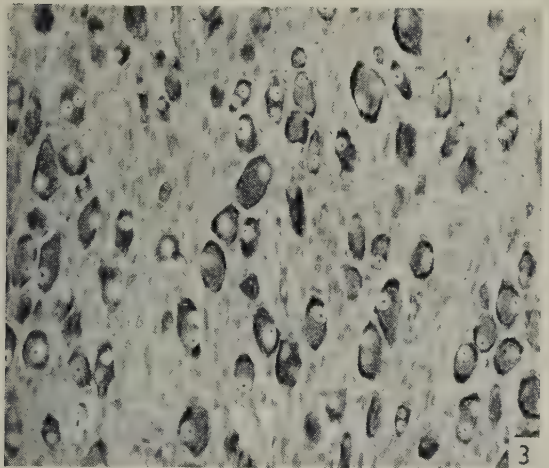
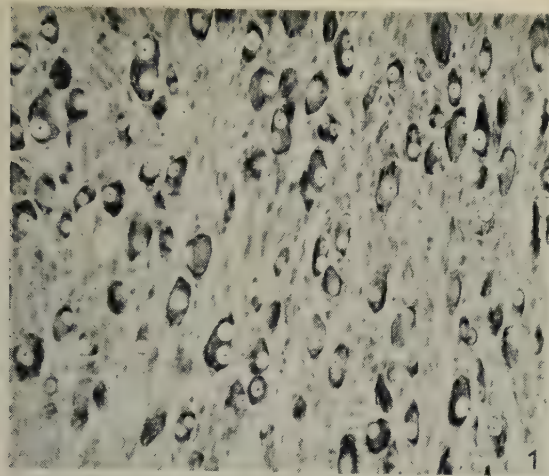
normal, and the mean cross-sectional area of the neurons was reduced. There was an increase in the connective tissue of the ganglion.

3. The interpretation of the morphological changes which take place in deafferented neurons is difficult. The absence of the normal stimuli may cause alterations in the neuronal metabolism, but it is likely that the change in the immediate environment of the cell caused by the disintegration of the afferent terminals is an important factor.

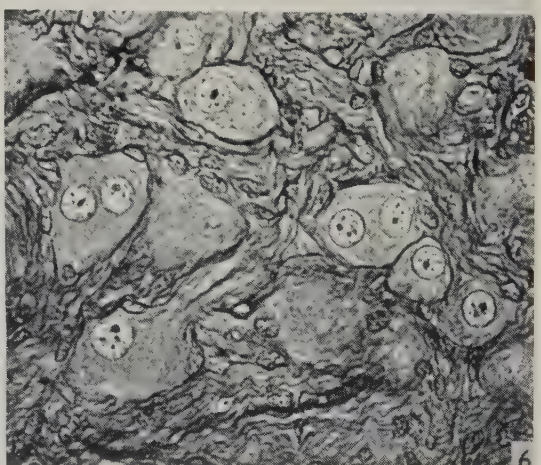
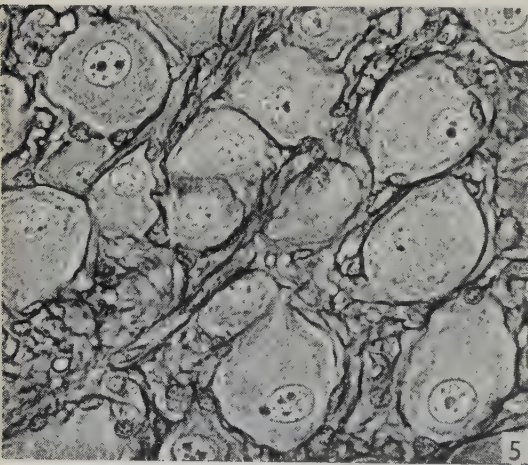
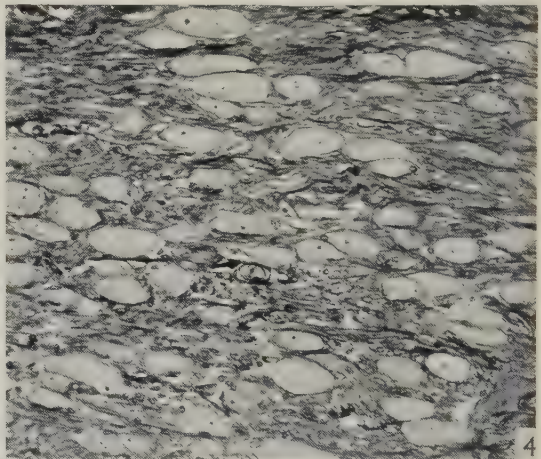
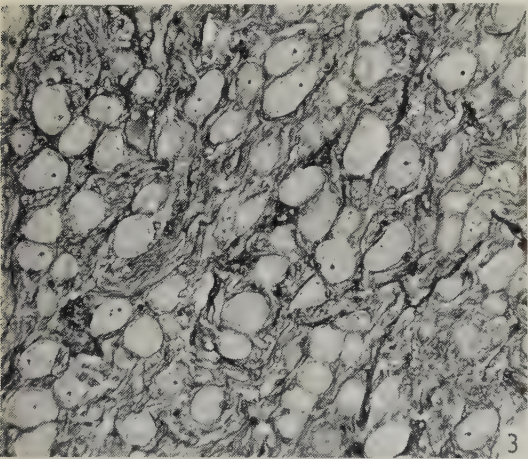
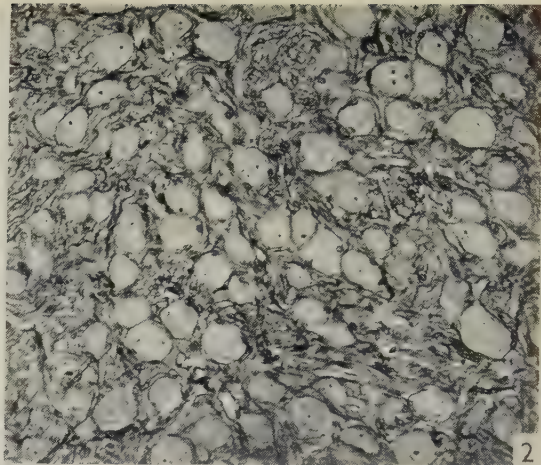
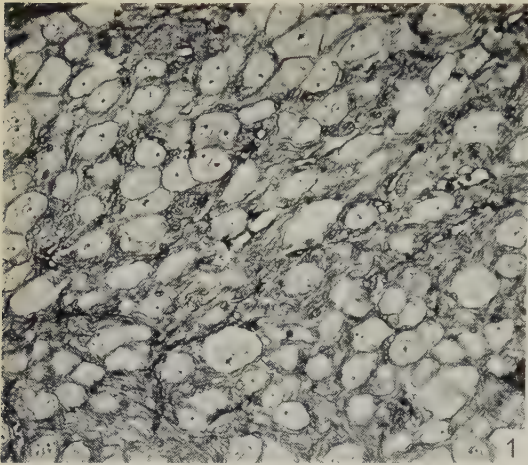
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## EXPLANATION OF PLATES

### PLATE 1

- Fig. 1. (Rabbit 219.) Nissl preparation of the superior cervical ganglion of the control side.  $\times 160$ .
- Fig. 2. (Rabbit 219.) Nissl preparation of the superior cervical ganglion of the operated side 40 days after isolation.  $\times 160$ . The Nissl substance is paler than that seen in the control.
- Fig. 3. (Rabbit 221.) Nissl preparation of the superior cervical ganglion of the control side.  $\times 160$ .
- Fig. 4. (Rabbit 221.) Nissl preparation of the superior cervical ganglion of the operated side 80 days after isolation.  $\times 160$ . There is increased pallor of the Nissl substance.
- Fig. 5. (Rabbit 218.) Nissl preparation of the superior cervical ganglion of the control side.  $\times 440$ .
- Fig. 6. (Rabbit 218.) Nissl preparation of the superior cervical ganglion of the operated side 40 days after isolation.  $\times 440$ . The Nissl substance does not stain so strongly as that of the control ganglion.

### PLATE 2

- Fig. 1. (Rabbit 220.) Azo-carmin and aniline-blue preparation of the superior cervical ganglion of the control side.  $\times 160$ .
- Fig. 2. (Rabbit 220.) Azo-carmin and aniline-blue preparation of the superior cervical ganglion of the operated side 40 days after isolation.  $\times 160$ . There is a slight increase in the amount of connective tissue.
- Fig. 3. (Rabbit 222.) Azo-carmin and aniline-blue preparation of the superior cervical ganglion of the control side.  $\times 160$ .
- Fig. 4. (Rabbit 222.) Azo-carmin and aniline-blue preparation of the superior cervical ganglion of the operated side 80 days after isolation. Some increase of connective tissue may be seen.
- Fig. 5. (Rabbit 223.) Azo-carmin and aniline-blue preparation of the superior cervical ganglion of the control side.  $\times 440$ .
- Fig. 6. (Rabbit 223.) Azo-carmin and aniline-blue preparation of the superior cervical ganglion of the operated side 80 days after isolation.  $\times 440$ . An increase of the connective tissue may be seen.

# THE EFFECT OF FIXATION ON NEURONS OF THE CHICK

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## INTRODUCTION

The literature relating to the intimate texture of the nervous system is certainly as large as in any other branch of histology or cytology. Some of its fundamental problems, however, such as the nature of neurofibrillae, and the action of fixatives on the neurocytoplasm, are still being debated without any immediate prospect of a final conclusion. The first of these topics is the older, and goes back as far as Remak (1844) and Parker (1929), before the use of fixatives in microscopy became general. From that time, there have been occasional cytologists such as Schultze (1870) and Held (1895) who studied the unfixed nerve cell which at the present time is again receiving attention. Furthermore, attempts at fixation by freezing, a method perfected only in recent years, were made as early as 1874 (Key & Retzius). Thus it may be said that comparison of the appearance of the living cell with that produced by various methods of preparation has been from time to time attempted, but that this approach has mostly been submerged by the development of particular techniques such as silver impregnation methods, and in recent years, electron microscopy, which are relevant only to fixed material. At the present time, however, advances in optics applicable to the living cell and to unstained preparations, such as phase-contrast and ultra-violet microscopy, have greatly increased the scope of this general approach and an increasing number of workers are at present taking advantage of these possibilities (Thomas, 1947; Koenig & Feldman, 1953; Adamstone & Taylor, 1953; and Taylor & Adamstone, 1953). My own interest in this field began with the observation of cell bodies of chick embryonic spinal ganglia in tissue culture. Under the phase-contrast microscope they have a granular texture

(Hughes, 1953), although a fibrillar appearance in them with ordinary microscopy has previously been described (Weiss & Wang, 1936; and Levi & Meyer, 1937). A comparable granular appearance was then observed in other immature unfixed neurons of the chick, although standard neuro-histological procedures on the same material produced the usual appearance of neurofibrillae. Experiments which have attempted to explain these discrepancies are here described.

#### TECHNIQUE

##### (1) *Observation of living neurons in dorsal root ganglia*

Spinal ganglia were dissected from 10- to 12-day chick embryos under sterile conditions and were explanted as hanging-drop cultures into a medium consisting of roughly equal parts of fowl plasma and chick embryo extract. In such cultures, Schwann cells migrate outwards in a fibroblastic form of outgrowth, accompanying the regenerating neurites. Many of the neurons within the explant degenerate, but in favourable cultures the remainder form a thin layer in the centre, which are accessible for microscopic observation after most of the Schwann cells have left. Usually, nearly a week of incubation is necessary before this stage is reached. Recently, however, at the suggestion of Dr H. B. Fell, it has been found that short treatment of the ganglia with 1% trypsin in Tyrode saline before explantation digests the intercellular matrix (Moscona, 1952) sufficiently to accelerate the separation of its constituent cells in culture, so that the cell bodies can often be observed much sooner after explantation than would otherwise be possible. Murnaghan (1941) found preliminary treatment with trypsin of use in making spreads of spinal ganglia.

##### (2) *The squash method for the cerebellum*

If fragments of the cerebellum of a chick are squashed between a slide and cover-slip, and then examined under the phase microscope, Purkinje cells may be examined unfixed. They are distorted in form by the treatment and their processes are shorn off; nevertheless, the nucleus and cytoplasm of the cell body may be studied for a period of 15 min. or so without showing any degenerative changes. The method is applicable to chicks either before or after hatching. In the squashed preparation, it is necessary to search for the Purkinje cells amongst a jumble of fibres and smaller cells, and so the method is only suitable for nervous tissues in which one type of neuron is present in very large numbers. This technique can also be used for the detection of the first appearance within the central nervous system of myelinated fibres which are evident in the chick cerebellum after 18 days' incubation. At later stages, however, when myelination is far advanced, the presence of so much material of high refractive index completely obscures cellular detail elsewhere in the preparation.

The method can be used for the study of the effects of some fixatives on the texture of neurocytoplasm, by squashing a fragment of the cerebellum in a drop of a particular reagent. Protein precipitants, however, cause such marked increase in the refractive index of the cell constituents, that the use of this technique is mainly restricted to formalin and osmic acid which fix by other chemical processes. After fixation for a few minutes in either of these reagents, the preparation is washed in



distilled water, mounted in glycerol and then observed in the phase microscope. In order to retain the film of squashed material adherent to the cover-slip during these operations, it is advisable first to place on the microscope slide a small square of wet cellophane and to squash the fragment initially between this and the cover. This 'sandwich' of cover-slip, tissue and cellophane may then with care be slid off the slide and the cellophane peeled off in water. The film of squashed nervous tissue should then remain on the cover-glass during any subsequent operations. Staining of such films is thus possible.

### (3) *Fixation by freeze-drying*

Dr Bell of the Zoology Department of King's College, London, was kind enough to undertake fixation by this method for me. Fragments no larger than 2 mm. across were dissected out of the cerebellum of chicks either before or within a few days after hatching. These were placed on aluminium foil and quenched in *isopentane* at a temperature near 160° C. They were then dehydrated at -40° C. in the apparatus of Messrs Edwards designed by Prof. J. F. Danielli (Bell, 1952; Danielli, 1953). The specimens were then infiltrated with paraffin wax for a few minutes and were subsequently treated at Cambridge. Sections were cut at 8 $\mu$  and mounted on clean slides thinly coated with celloidin. Frozen-dried material must be kept from contact with water, as the proteins therein have not yet suffered denaturation. For this reason the sections must be flattened in a non-aqueous medium in which paraffin wax does not dissolve. At the suggestion of Dr Bell, acetonitrile was used for this purpose.

Some slides of these sections were de-waxed in xylol and mounted in liquid paraffin for observation directly by the phase microscope. Others were first dipped in absolute alcohol to denature their proteins, then in 1% celloidin in absolute alcohol and ether to retain the sections on the slide and afterwards in 70% alcohol to harden the resulting celloidin film. They were then ready for staining by neuro-histological methods.

The inevitable use of small fragments for freeze-drying meant that the sections were orientated at random on the slide. It was thus necessary to prepare a number of fragments and to select sections which happen to lie in the desired plane.

### (4) *Chemical fixation*

Chick cerebella were fixed whole either in formol-saline or in Carnoy's mixture for 24 hr., or in absolute alcohol with 1% ammonia for 48 hr. when Ranson's method of silver impregnation was being used (Ranson, 1914). Before fixation in 2% osmic acid, the cerebellum was cut into fragments about 1 mm. square and left therein for 24 hr.

### (5) *Staining methods*

The impregnation technique which was mainly used is that of Holmes (1947) in which mounted sections are silvered. With the chick cerebellum, it was effective either after fixation by freeze-drying, or in formol-saline or Carnoy's fluid. The method is also applicable to cultures of spinal ganglia. After fixation in osmic acid, although the cell bodies are coloured brown by silver, their fibres do not then take up the

metal. Ranson's method (Ranson, 1914) was also used on the cerebellum for purposes of comparison.

For staining Nissl substance, thionin was used in a buffer at approximately pH 3.7 as recommended by Windle, Rhines & Rankin (1943). A formate buffer was found satisfactory for the purpose when a solution of the dye (0.5 %) rather stronger than that recommended by these authors was found advisable. It was possible to combine in the same sections a light silver impregnation with subsequent staining of the Nissl substance.

#### (6) *Phase-contrast microscopy*

The living cultures of spinal ganglia or the unstained preparations of cerebellar squashes or of frozen-dried sections were studied with a Cooke, Troughton and Simms phase-contrast microscope, usually under the  $\times 95$  oil-immersion objective. To record these observations, photo-micrography is essential, and a rapid technique is desirable with evanescent preparations such as squashed unfixed films. A strong light source is required; the 250 W 'Mercra' high-pressure mercury arc of Messrs B.T.H. was used for the purpose with a Wratten mercury monochromatic filter no. 77. Exposures were made on Ilford 'Micro-Neg' Pan film by means of a 35 mm. reflex camera mounted over the microscope. In enlarging these negatives on to Bromide paper, it was often found necessary to use the most contrasty grades of paper.

### RESULTS

#### (1) *Comparison of different methods of preparation and observation*

A generally granular texture is revealed by the phase microscope in the cytoplasm of neurons of the chick which have not been treated with chemical fixatives. This is true equally of living cells in cultures of spinal ganglia (Pl. 1, fig. 2) (Hughes, 1953, plate II, fig. 5); of unfixed Purkinje cells in squashes of the cerebellum (Pl. 2, figs. 9, 10); and in sections of the latter fixed by the freeze-drying technique. Probably more than one type of cell inclusion is represented by these granules, though some of this material is capable of impregnation with silver (Pl. 2, fig. 11).

Their form, size and arrangement differs in the different types of neuron. The finest granules of this type are seen in spinal neurons (Pl. 1, fig. 2), while the coarsest are those of the dentate nucleus of the cerebellum (Pl. 1, fig. 6). In squashed Purkinje cells, the granular inclusions of all types are dispersed to some extent (Pl. 2, fig. 9), while in sections of the frozen-dried cerebellum, they are tightly packed (Pl. 2, figs. 10, 11). They are arranged at random in the main body of the cell but tend to be orientated within the dendrite. Here the individual elements may take the form of short rod-like bodies (Pl. 2, fig. 10). After silver impregnation the appearance of the frozen-dried Purkinje cell is similar to that which it previously exhibited under the phase microscope, though some granules take up silver more heavily than do others. There is a slight tendency for their coalescence into short rows which in the dendrite stem are linearly arranged (Pl. 2, fig. 11). Here, this orientation is shared by particles of the Nissl substance, as seen in preparations stained with thionin (Pl. 2, fig. 13).

After fixation of the cerebellum in formol-saline or Carnoy's fluid, followed by silver impregnation by Holmes's method, occasional granules are still separate, but

the majority have formed continuous chains in some of which the constituent granules can be recognized (Pl. 2, fig. 12). The distortion which is caused by chemical fixation is even more strikingly seen within the nucleus, for the nuclear contents often become aggregated around the nucleolus, with an empty space between this whole mass and the nuclear membrane. This condition is in marked contrast to that in the frozen-dried neuron, where the nucleolus is discrete, with the rest of the nucleus filled with nucleoplasm, within which a few small chromocentres can be seen. Cell shrinkage is still greater after fixation in the absolute alcohol-ammonia mixture which is used in the Ranson method of silver impregnation. In such preparations, none of the impregnated substance within the cytoplasm is present as separate granules, and there are clear spaces between the chains of silvered material. In frozen-dried preparations, the neurons of the dentate nucleus contain argyrophil granules larger and fewer in number than those of the Purkinje cells (Pl. 1, fig. 6). In chemically fixed material, these coalesce completely into fibrils arranged in the reticular pattern characteristic of conventional illustrations of the neuron.

In the cell bodies of living ganglionic neurons in culture, more than one type of granule can be seen. Around the nucleus is a zone containing short rod-like bodies, which have been identified by Murnaghan (1941) as mitochondria. Elsewhere, the phase microscope reveals much smaller rounded granules, best seen at focal levels above and below that of the nucleus. Although continuous lines of neurofibrillar material are not to be seen within the cell body, yet in the neurite stem there can occasionally be observed a faint parallel striation (Pl. 1, fig. 2) distinct from the elongated mitochondria which Murnaghan found therein.

In the neurons of spinal ganglion cultures fixed in formalin and impregnated by Holmes's method, a bundle of neurofibrillae can be traced in the neurite stem (Pl. 1, fig. 3). They fan out as they enter the cell body, in the rest of which they are much less definite. There they form a rather vague reticulum on which little silver is deposited. After fixation in Carnoy's fluid, impregnation reveals a different picture, for the neurofibrillae then form an extremely coarse network, heavily loaded with silver. Other neurons in such preparations show a different and still more severe form of shrinkage; in them the cytoplasm is contracted to a small totally blackened knot, from which the nucleus bulges on one side.

The appearance of the neurofibrillar material in impregnated preparations, then, depends entirely on the method of fixation employed. The distribution and arrangement of the other main cytoplasmic constituent of the neuron, the Nissl substance, is known to be similarly affected by fixation. In sections of the frozen-dried cerebellum, the great bulk of this material is found as large amorphous and flocculent masses (Pl. 2, fig. 13). After fixation in Carnoy's fluid the Nissl substance has a generally reticular arrangement (Pl. 2, fig. 14). Its appearance in osmic-fixed squashes and spinal ganglion cultures is intermediate between these two conditions. The total amount of Nissl substance is much greater after freeze-drying; Dr Bell informs me that it is generally true that this method of treatment preserves a much greater degree of basophilia in other cells than is ordinarily found in chemically fixed material.



*(2) The effect of chemical fixatives*

Since the cytoplasmic texture of these neurons is so much affected by standard neuro-histological procedures, it is necessary to ask at what stage these changes originate. Observation of the action of chemical fixatives suggests that this first step is the one mainly responsible.

When a fragment of the fresh chick cerebellum is squashed in 5% neutral formalin and the squash mounted in glycerol after washing in water, the cytoplasm of the Purkinje cells under the phase microscope is then seen to be uniformly filled with vacuoles up to  $2\mu$  in diameter (Pl. 2, fig. 16). When 1 or 2% osmic acid is used, and the procedure repeated, the vacuoles are smaller, but are still uniformly distributed throughout the cytoplasm (Pl. 2, fig. 15).

If cultures of spinal ganglia are fixed in formalin and mounted in glycerol, vacuoles are also seen within the cytoplasm of the neurons, but are now as small as those produced in the Purkinje cell by osmic acid (Pl. 1, fig. 1). In silvered preparations of the same material fixed with the same agents, the meshes of the network of the neurofibrillae are roughly proportional in size and distinctness to the diameter of the vacuoles formed by the fixatives alone. Neutral formalin produced a loose network in the Purkinje cells, but a fine and rather vague one in spinal ganglion cultures; while after fixation in osmic acid, Purkinje cells do not exhibit a network, though here, the fixative considerably modifies the impregnation process.

This correlation suggests that the network of the fixed neuron is formed between vacuoles. In other cells, as Hardy (1899) showed, a fine network may be formed at fixation, though in such meshworks the interstices are too small to be distinguishable as vacuoles. When, however, these are formed within a cell at fixation, much of its water must be withdrawn from the cytoplasm; as the volume of the cytoplasmic phase decreases, particles suspended therein must be concentrated together. In the neuron, these changes confer a spurious definiteness on both the Nissl substance and the neurofibrillar material.

If this explanation is correct, then the interstices between the network of silvered preparations should be empty spaces in the sections, not occupied by cytoplasmic ground substance, or by any other cell constituent. This point has been checked under the phase microscope in mounted sections of the cerebellum fixed in formol saline, de-waxed but unsilvered. In such preparations, within the cytoplasm of Purkinje cells and of the neurons of the dentate nucleus, there are to be seen empty spaces corresponding to the interstices of the silvered network, though these spaces lack the sharp boundaries of the vacuoles in squashed preparations, for which dehydration, the extraction of lipoids and shrinkage in the course of preparation for sectioning may be responsible.

The formation of vacuoles within cells after fixation in formalin has already been described by Crawford & Barer (1951). In the neurons of the chick, however, they are produced in much greater numbers throughout the whole cell body. To establish any general validity for this hypothesis of network formation, it would be necessary to show that in general, neurons are specially susceptible to the formation of vacuoles on chemical fixation. Similar vacuoles were evident in the Purkinje

cells of the adult human cerebellum fixed in formalin, sectioned by freezing, and examined in the phase microscope.

#### DISCUSSION

The effect of chemical fixatives on the texture of the neurocytoplasm has been studied repeatedly, though in nearly all such studies the form of the Nissl substance has alone been considered. In the year following Nissl's description of the chromophilic granules of the nerve cell (Nissl, 1894), Held (1895) published a classical paper in which the effect of vacuolation on the form of the Nissl substance was clearly demonstrated. Held isolated fresh anterior horn cells from the spinal cord of the guinea-pig and studied them first unfixed but mounted in saline. This treatment by itself produced cytoplasmic vacuolation which was much exaggerated by treatment with water alone. After staining, it was seen that the Nissl material had been distorted into a reticulum whose meshes surrounded these vacuoles.

In 1911 Møllgaard attempted to avoid fixation artefacts by freezing, though at temperatures near enough to 0° C. for the formation of ice crystals of appreciable size between which a fine network of neurocytoplasm was produced. This artefact of the freeze-drying method which even with modern technique can appear in fragments of tissue too large for instantaneous quenching, had already been observed by Key & Retzius (1874). The effect of different chemical fixatives in producing various degrees of aggregation of the Nissl substance has since been studied by Hopkins (1924) and by Sheinen (1932). The form of the Nissl material in neurons fixed by modern freeze-drying technique has been demonstrated by Bensley & Gersh (1933) and by Hyden (1943). Living neurons of chick embryos in culture have been photographed in the ultra-violet by Koenig and Feldman (1953) who find 'that in the cytoplasm, most of the absorbing material is homogenous and unresolved'.

Although the effect of fixatives on the form of the Nissl material has been well investigated, there have hitherto been no comparable studies on the fixation artefacts of the neurofibrillae, even though these structures have been repeatedly studied in the perikaryon of the living neuron (Remak, 1844; Schultze, 1870; Weiss & Wang, 1936; Levi & Meyer, 1937; Murnaghan, 1941; and Thomas, 1947). Several other authors have described neurofibrillae of living nerve fibres, but here it will be as well to discuss separately the fibrillae in the cell body and in the nerve process.

Remak's paper in 1844 was the first to describe neurofibrillae; within a neuron from the ventral ganglionic chain of the crayfish he illustrates an arrangement of striae round the nucleus which follows the curve of the cell wall. Schultze (1870) gives a remarkable drawing of a ganglion cell isolated from the electric lobe of the brain of *Torpedo*. As each nerve process joins the perikaryon, parallel neurofibrillae fan out towards the nucleus, round which a number of concentric lines are also shown. The photomicrograph of living neurons from a culture of a spinal ganglion of the chick given by Weiss & Wang (1936) again shows both fibrillae round the nucleus and also parallel bundles within the neurites, all of which are considered by Murnaghan (1941) to be mitochondria. In none of these examples, moreover, is neurofibrillae shown in the reticular pattern characteristic of fixed and impregnated preparations. The first observer to use the phase-contrast microscope on a living

neuron was Thomas (1947), who examined cell bodies of the snail in which, by this method, he could see a very long chain of minute 'cocci'. This chain was looped round the nucleus with the two ends continued into the axon. In his preparations, Thomas examined the same field with and without phase contrast and stated that 'with ordinary transmitted illumination they [the "cocci"] cannot with certainty be recognized; but if a quick transition from transmitted to phase-contrast illumination be made on the same field, the impression of a very faint striation at once gives place to the well-defined picture'.

During the course of the present work, a similar comparison with ganglionic neurons of the chick in culture led to much the same conclusion. Sufficient contrast to examine or photograph a living cell body by the ordinary microscope is only obtained when the substage aperture is so far reduced that diffraction effects become prominent; when alternate light and dark bands parallel to surfaces of discontinuity such as the cell and nuclear membranes are produced. Hence by phase contrast the cytoplasm of the perikaryon of these neurons has a granular texture, but otherwise a more fibrillar effect is seen. It is thus advisable to regard with caution any appearance of parallel striations in living neurons unless and until the phase microscope has also been used to examine the same material. That there is ample scope for further research of this kind is at once obvious from a glance at the original figures of living neurons given by Remak and Schultze.

In living nerve fibres, however, it is very unlikely that the apparent neurofibrillae are always merely optical artefacts, for some of them at least do not run parallel with the surface membrane. Those of the nerves of the crayfish, for instance, which Remak (1844) originally described, have a wavy course within a straight fibre. Again, in the present work, one instance was seen of a chick ganglion cell in culture where, by phase contrast, a continuous neurofibril was seen lying obliquely across the course of a neurite entering the cell body (Pl. 1, fig. 2). In sections of the cerebellum fixed by freeze-drying, much the same distinction was clearly seen within Purkinje cells between the arrangement of the neurofibrillar material in the dendrite and in the perikaryon. Cowdry (1913), in his remarkable study of the effect of various fixatives and stains on the spinal ganglia of the adult pigeon, shows figures in which parallel neurofibrillae, stained by various methods but without silver impregnation, are restricted to the axon alone.

This difference in form of the same cytoplasmic material in cell body and nerve fibre is incompletely understood. It is shared also by other cell constituents, such as the mitochondria (Nicholson, 1916; and Murnaghan, 1941) and the Nissl material (Pl. 2, fig. 13). Smith (1952) finds that in sympathetic ganglionic neurons of the toad, homogeneous protein-containing droplets are spherical within the perikaryon, but are elongated in the axon hillock. Chambers & Kao (1951) find that oil drops and air-bubbles micro-injected into the gelated cortex of giant nerve fibres of the squid assume ovoid shapes, which these authors ascribe to the influence of a linearly arranged protoplasmic ultra-structure. This, however, has been shown to be qualitatively similar in some neurons in both cell body and nerve fibre by Chinn (1938), who studied the birefringence of living neurons from the leech and the frog.

Within recent years, the nerve fibre alone has been considered in nearly all those



papers which have dealt with neurofibrillae. Hoerr (1936), in the only previous work in which freeze-drying fixation has been applied in this field, found evident neurofibrillae in rabbit axons fixed in this way, either with subsequent silver impregnation or treated with osmic vapour. In most of the recent studies by means of the electron microscope on the nerve fibre, filamentous bodies have been found therein, usually beaded in form (Schmitt, 1950; Fernandez-Moran, 1950; and Duncan, 1951) though opinion is not unanimous on this question. Pease & Baker (1951) concluded that 'neurofibrils in the conventional sense are artefacts of fixation. When fixation is optional, coarse fibrillar structures are to be seen only in the immediate vicinity of the nodes of Ranvier in peripheral axones'. It is evident that not only do different authors have varying concepts of what constitutes a neurofibril but also that the state of aggregation of neurocytoplasmic material into fibrillae is not the same in all nerves nor, moreover, as the present work suggests, is it necessarily identical in cell body and nerve fibre in the same neuron. Questions of definitions and nomenclature must therefore be decided as further work in this field proceeds. Argyrophil elements within neurocytoplasm may be granular or fibrillar in form; they are thus not satisfactorily described by the term 'neurofibrillae'. This word would better be kept strictly for the impregnated filaments of the silvered preparation, with the implication that their form, to a varying extent, is of the nature of an artefact.

#### SUMMARY

1. The appearance has been compared of neurons from the chick prepared for microscopical observation in various ways.

2. In the cerebellum, Purkinje cells and the neurons of the dentate nucleus, have been studied at or near the hatching stage. Neurons in spinal ganglia of 12-day embryos have been observed in tissue culture.

3. Ganglionic neurons have been studied alive, and unfixed Purkinje cells observed in squashes. Fixation both by freeze-drying and by common chemical fixatives has been employed. The appearance of unstained preparations by phase microscopy has been compared with the effect of methods of silver impregnation.

4. The cytoplasm of the perikaryon either unfixed or frozen-dried has a granular texture. The granules are either rounded or rod-like, and vary in their affinity for silver. In nerve processes, they tend to be longitudinally arranged.

5. Chemical fixatives cause vacuoles to form within the cytoplasm of the neuron. They vary in size with different fixatives, and with different neurons. When large enough, the vacuoles distort the cytoplasm into a network. It is suggested that the typical reticular arrangement of neurofibrillae within the perikaryon in neuro-histological preparations is produced by this means.

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### EXPLANATION OF PLATES

All figures are photomicrographs of the cell bodies of neurons from chicks either embryonic or within 2 days after hatching. Unstained material photographed by 2 mm. phase-contrast objective; stained preparations by 2 mm. apochromatic objective N.A.1.30.

#### PLATE 1

Figs. 1-4 are of unipolar neurons from cultures of spinal ganglia of 12-day chick embryos, grown for 3-6 days *in vitro*;  $\times 1500$ .

Fig. 1. Fixed in 5% neutral formalin and mounted in glycerol. Very fine vacuoles are present throughout the cytoplasm.

Fig. 2. Phase contrast. Living culture mounted in liquid paraffin. In the neurite, fine neurofibrillae run somewhat obliquely.

Fig. 3. Fixed in 5% neutral formalin and silvered by Holmes's method. Neurofibrillae most distinct in neurite; in cell body, light deposit of silver on fine reticulum.

Fig. 4. Fixed in Carnoy's fluid and silvered by Holmes's method. Heavy silvering of coarse reticulum.

Figs. 5-6 are of neurons from the dentate nucleus in sections of the cerebellum of chicks near or soon after hatching;  $\times 2200$ .

Fig. 5. Phase contrast. Fixed in 2% osmic acid. Sections mounted in D.P.X. without further staining. Dense granular texture of cytoplasm.

Fig. 6. Fixed by freeze-drying, and silvered by Holmes's method. Argyrophil material consists of granules and short filaments forming an incomplete reticulum.

Fig. 7. Fixed in Carnoy's fluid and silvered by Holmes's method. Reticulum of impregnated fibrils more definite than in fig. 6. Note shrinkage of nuclear contents.

Fig. 8. Fixed in alcohol-ammonia and silvered by Ranson's method. Coarse network of impregnated fibrils.

#### PLATE 2

Figs. 9-16 are of Purkinje cells from the cerebellum of chicks near or soon after hatching;  $\times 2000$ . Reduced 5 = 4.

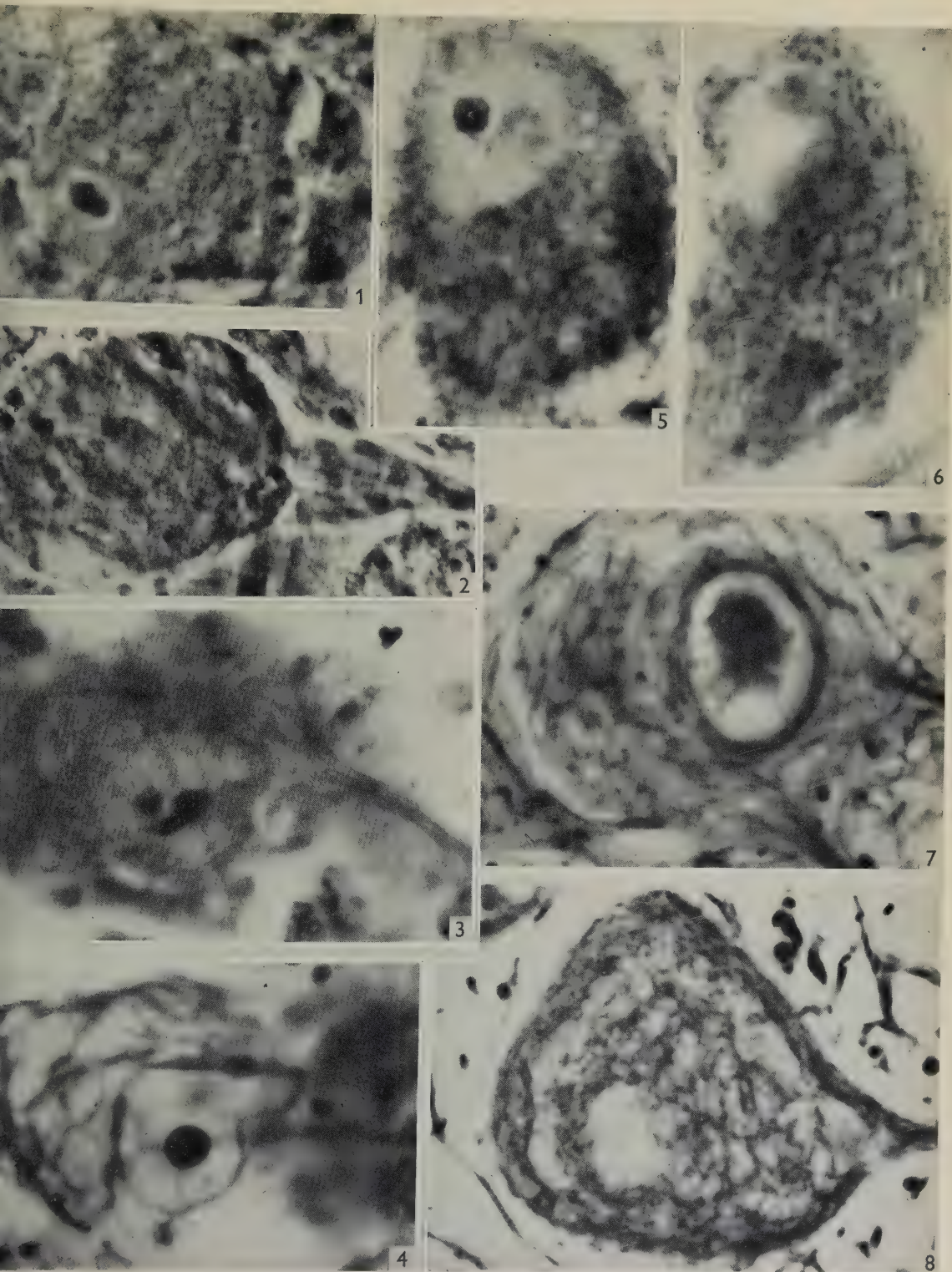
Fig. 9. Phase contrast. Unfixed squashed preparation. Within the cytoplasm are seen granules or short filaments, which tend to an orientated arrangement towards the dendrite.

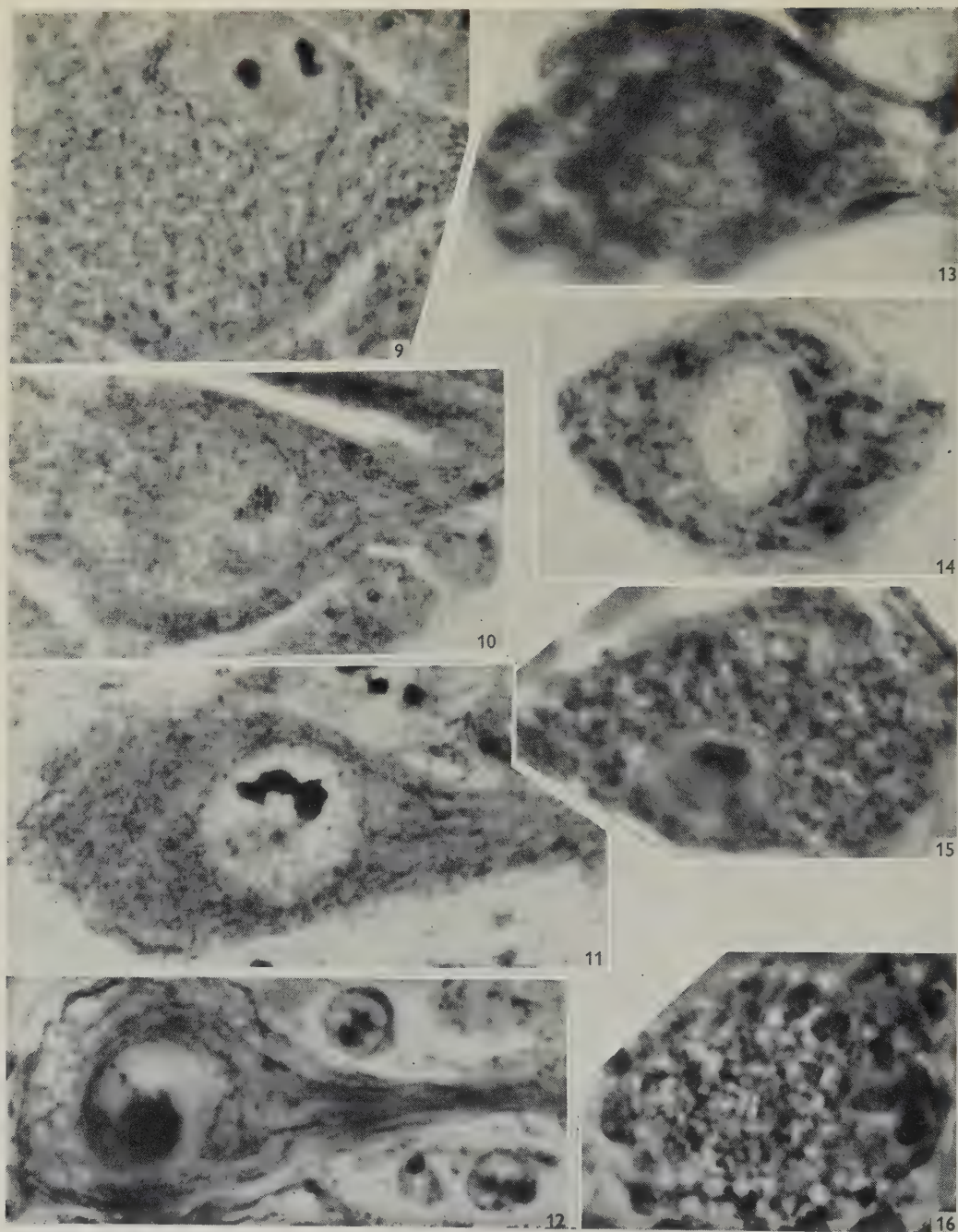
Fig. 10. Phase contrast. Fixed by freeze-drying, sectioned and mounted in liquid paraffin. Granular texture in cytoplasm, with linear arrangement towards dendrite.

Fig. 11. Fixed by freeze-drying, sectioned and silvered by Holmes's method. Similar texture to fig. 10; some granules, however, are more argyrophilic than others.

Fig. 12. Fixed in Carnoy's fluid and silvered by Holmes's method. Network of argyrophil material in cell body; straight filaments in dendrite, which has shrunk in diameter. Also notice shrinkage of nuclear contents.







HUGHES—THE EFFECT OF FIXATION ON NEURONS OF THE CHICK



- Fig. 13. Fixed by freeze-drying and stained in buffered thionin. Nissl substance mainly floccular in texture with tendency towards linear arrangement in dendrite.
- Fig. 14. Fixed in Carnoy and stained in buffered thionin. Nissl substance precipitated into definite filament, partly reticular in arrangements.
- Fig. 15. Phase contrast. Squashed in 1 % osmic acid. Cytoplasm uniformly filled with vacuoles  $1\mu$  or less in diameter. Note thickening of nuclear membrane.
- Fig. 16. Phase contrast. Squashed in 5 % neutral formalin. Cytoplasm filled with larger vacuoles, some of which are more than  $2\mu$  in diameter. Nucleus pushed to extreme right of cell.



# THE MORPHOLOGY OF THE COLLATERAL CIRCULATION FOLLOWING COMPLETE INTERRUPTION OF THE ABDOMINAL AORTA IN THE RAT

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Astley Cooper (1818) is credited as the first observer to carry out *acute* interruption of the abdominal aorta both in man and the experimental animal (dog), although observations on the effects of *temporary* occlusion of the aorta in the rabbit were made as early as 1669 by Stenon.

The pioneer work of Cooper was followed by other attempts to ligate the aorta in man, the indications being aneurysm either of the terminal part of the aorta or the common iliac arteries. James (1830), Murray (1834) and Monteiro (1842) were among the first to describe in varying detail their individual operative techniques and post-mortem findings in patients undergoing this surgical hazard. These early failures fostered a good deal of experimental work, both on human post-mortem material and, more important, on the living animal. Many different species of animal have been subjected to acute ligation, the most common being the dog and rabbit. The type of operation has varied from an acute interruption to all types of gradual occlusion. The only feature common to the many and varied techniques rests in their end results, which were published mainly in the form of post-mortem reports. Thus Porta (1845), whose researches on the effects of large vessel occlusion were the most comprehensive, recorded only five survivals out of sixty animals subjected to aortic ligations of various types. Pirogoff (1838), Kast (1880), Offergeld (1907), and more recently Owings & Hewitt (1942), have recorded equally high mortality rates.

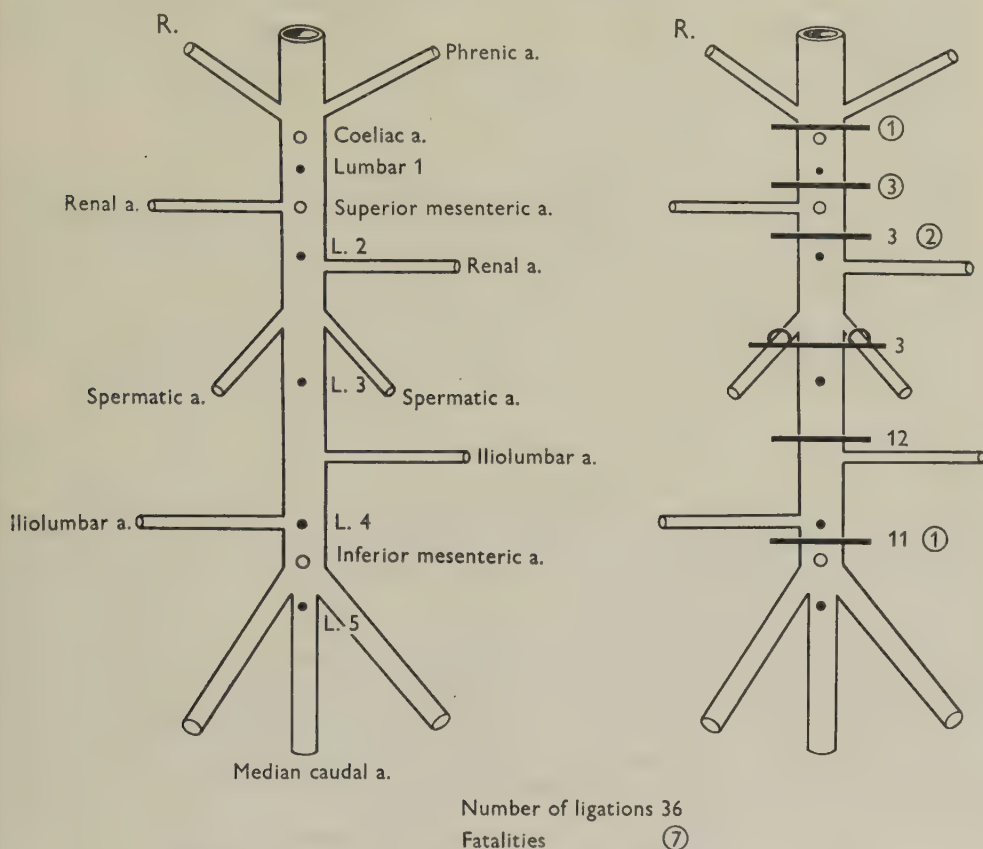
The literature which has accumulated on aortic ligation, though large is thus concerned mainly with the post-operative sequelae, and the high mortality rate has considerably limited the number of observations on the morphology of the collateral circulation following this procedure.

Whilst studying the effect of ligation of the pelvic arteries on the viability of the bladder in the rat (Braithwaite, 1953), it was noted that even occlusion of both common iliac arteries results in no fatal complication. It seemed reasonable that this animal would probably withstand ligation of the terminal part of the abdominal aorta just proximal to its bifurcation, and pilot experiments confirmed this view. It was felt that this animal would be more suitable than others used previously for the study of the morphology of the collateral circulation in a large series of experiments.

## MATERIALS AND METHODS

Rats of both sexes were used, although the majority were female; their body weights ranged between 150 and 350 g. Twenty observations were made on the topography of the abdominal aorta, noting particularly the site of origin of its branches and any

variations (Text-fig. 1). The preformed anastomoses occurring between the branches of the aorta themselves, and also between the latter and other arteries arising from more remote sources, were also noted.



Text-fig. 1. The sites of occlusion of the abdominal aorta in the rat.

The abdominal aorta was interrupted in thirty-three animals at levels varying from a point proximal to the origin of the coeliac artery to just proximal to the bifurcation (Text-fig. 1). In three animals interruption of the aorta was combined with the additional section of the spermatic anastomoses. The ligation was either single or double, although in a single instance the vessel was divided between two sutures.

The surviving animals were sacrificed at periods from 5 min. to 3 months after operation, and injected through the thoracic aorta with a radiopaque medium.

#### *Injection technique*

'Chlorbismol' (May and Baker) employed initially was replaced by 'Micropaque' (Damancy and Co.); the former passed freely into the venous system, and although ligation of the renal pedicles prior to injection prevented much of the overspill, this was felt to be undesirable. The success of the injection was assessed by delivering

a loop of gut through a small midline abdominal incision and noting the filling of the finest radicles. The amount of medium introduced averaged about 5 ml. The time interval between injection and radiography was considered to be a most important factor. Pilot radiographs taken at varying lengths of time after injection differ, mainly due to the gradual escape of the mass from the larger to the smaller vessels. Three standard radiographs were therefore taken in all instances:

- (1) With the gut in position (30 sec. after injection).
- (2) After delivering and retracting the gut (5 min. after injection).
- (3) After removal of the gut (20 min. after injection).

The interference involved prior to taking the third radiograph caused a variable amount of the injection mass to pass from the larger into the smaller vessels, but this was unavoidable. After radiography the specimens were later dissected and studied in conjunction with positive radiographs enlarged  $\times 2$ .

In certain cases tissues such as the body-wall and gut were removed, cleared by the Spalteholz method and compared with preparations from control animals similarly treated, to note more specifically the vascular patterns in these areas.

## FINDINGS

### *Topography of the rat aorta*

The origin of the main vessels shown in Text-fig. 1 is fairly constant; the most common variations being the occasional origin of one or other of the spermatic arteries from the renal arteries.

- (i) The right renal artery arises proximal to the left.
- (ii) The iliolumbar (transverse lumbar) arteries are constant branches of the aorta, the right vessel arising nearer the bifurcation than the left.
- (iii) The inferior mesenteric artery arises at the bifurcation of the aorta.
- (iv) The median caudal artery in the rat is a well developed vessel arising from the dorsal aspect of the aorta just proximal to the bifurcation.

### *Preformed macroscopic anastomoses*

The preformed macroscopic anastomoses observed by either radiography (Pl. 1, fig. 1), or dissection of the intact animal, which may potentially contribute to the collateral circulation following occlusion of the abdominal aorta at various levels are as follows:

#### (1) *Visceral anastomoses*

- (i) *Spermatic*. Between the ovarian and uterine arteries in the female (testicular and vasal arteries in the male).
- (ii) *Intestinal*. Between branches of the superior and inferior mesenteric arteries.
- (iii) *Ureteric*. Between the ureteric branches derived from adjacent arteries, notably the renal, spermatic and iliolumbar (or lumbar) arteries, and the aorta.
- (iv) *Renal*. Between the renal artery and renal capsular branches of the spermatic and adrenolumbar arteries.
- (v) *Spinal*. Between the anterior and posterior spinal arteries on the one hand and the intersegmental spinal branches of the posterior intercostal and lumbar arteries on the other.



(2) *Parietal*

(i) *Posterior body-wall.* Between contributions from the lower posterior intercostal, subcostal, lumbar, iliolumbar, deep circumflex iliac, and inferior phrenic arteries.

(ii) *Antero-lateral body-wall.* Between contributions from the superior and inferior epigastric arteries, lower anterior intercostals, subcostal and inferior phrenic arteries.

LIGATION EXPERIMENTS

The majority of the ligation experiments were carried out on the terminal part of the aorta, twelve just proximal to the bifurcation and twelve proximal to the origin of the iliolumbar arteries (Text-fig. 1); these sites were chosen because aneurysm most commonly affects either this segment of the aorta or its terminal common iliac branches. Of these twenty-four occlusions only one ended fatally, as compared with six fatalities out of nine operations at levels proximal to the left renal artery.

(1) *Ligations proximal to the bifurcation*

(a) *In an animal sacrificed within five minutes of operation*

A positive arteriograph from this animal shows that there is an absence of filling of the vessels below the site of ligation, whereas the branches of the aorta proximal to this level are well demonstrated (Pl. 1, fig. 2). Some of the injection medium has entered the superior left colic branch of the inferior mesenteric artery by way of its anastomosis with branches of the superior mesenteric artery.

(b) *In an animal sacrificed 24 hr. after operation*

The collateral vessels are beginning to open up at this stage. An arteriograph demonstrates the preformed macroscopic anastomoses which contribute to the collateral circulation (Pl. 1, fig. 4). These are the spermatic anastomoses of both sides, the mesenteric, and the right iliolumbar—deep circumflex iliac anastomoses.

(c) *In an animal sacrificed 10 days after operation*

Arteriographs taken with the gut retracted (Pl. 2, fig. 5), and after removal of the gut (Pl. 2, fig. 6), show more clearly the additional anastomotic channels.

The collateral vessels have undergone considerable enlargement compared with the previous experiment. The mesenteric and iliolumbar—deep circumflex iliac (Pl. 2, fig. 5), and the spermatic and lumbar anastomoses (Pl. 2, fig. 6), are all well developed.

(d) *In an animal dying within 24 hr. of operation*

In this instance the aorta was *divided* between two ligatures. An arteriograph taken after removal of the gut shows that the most striking feature is the marked retraction of the proximal segment of the aorta which has caused angulation of the branches arising from it, notably the left iliolumbar and right ovarian arteries (Pl. 1, fig. 3). Post-mortem examination revealed gangrene of the left uterine cornu, presumably caused by the cutting off of the blood supply through angulation of the left ovarian artery. The injection mass has not entered this vessel. There is only a moderate filling of the common iliac arteries distal to the site of section.

(2) *Ligations proximal to the origins of the iliolumbar arteries*

Although the latent interval between ligation of the aorta and the establishment of the collateral circulation is similar to that in the first group of experiments, the pattern of the fully developed anastomotic circulation differs slightly. An arteriograph taken 2 months after operation shows additional enlargement of the mesenteric anastomosis (Pl. 2, fig. 7), together with a well-marked plexus of ureteric vessels (Pl. 2, fig. 8). Enlargement of the body-wall anastomoses is also evident. The well-marked plexus of vessels in the antero-lateral body-wall, as a result of aortic ligation carried out 2 months before sacrifice, is well demonstrated (Pl. 3, fig. 10). When compared with a normal control (Pl. 3, fig. 9), there is considerable increase in the density of the vascular network, together with an increased tortuosity in the parent trunks contributing to the collateral circulation.

(3) *Ligations proximal to the origins of the iliolumbar arteries combined with section of the spermatic arteries*

This series comprised a group of three experiments designed to determine the effects of eliminating a part of the potential collateral circulation on the remaining anastomotic channels. It is evident from an arteriograph taken 3 months after operation, that the lumbar anastomoses and those occurring in the body-wall are larger than in any of the other series, and that they have compensated for the eliminated spermatic anastomoses (Pl. 3, fig. 11).

In addition to the enlargement of the preformed macroscopic anastomoses there is a localized network of extremely fine vessels around the ligation site. The contributions to this plexus arise from adjacent vessels. Thus when the aorta is ligated proximal to the iliolumbar arteries this 'para-aortic' plexus receives branches from the neighbouring spermatic, ureteric and lumbar arteries. The prominence of this plexus is more marked with the higher ligations.

#### DISCUSSION

In this series of experiments successful ligation was performed in twenty-nine out of thirty-six instances. All the fatalities, with one exception, occurred at levels proximal to the origin of the left renal artery. The survival rates compare very favourably with those of previous investigators using other experimental animals, and this has facilitated a more comprehensive study of the morphology of the collateral circulation. After a latent interval of about 4 days some enlargement is detectable in the preformed macroscopic channels, both the visceral and parietal anastomoses undergoing dilatation to a degree dependent on the site of ligation. Thus when the iliolumbar arteries are excluded from contributing to the collateral circuit by occlusion of the aorta proximal to their origin, further enlargement is seen in the vessels of the body-wall and along the ureter; the spermatic and lumbar channels also become more dilated. The additional elimination of the spermatic arteries causes still further enlargement of the remaining channels, particularly the lumbar anastomoses.

The present findings do not corroborate the work of Porta (1845), who used the dog, calf, cat and sheep as experimental animals; he considered that the visceral

anastomoses were of no importance in the establishment of the collateral circulation, neither do the results substantiate the conclusions of Pirogoff (1838) working on the dog, sheep, goat and rabbit, and Kast (1880) on the rabbit and dog. The latter stressed that the body-wall (epigastric) anastomoses were the most important contribution to the collateral network, and the former emphasized the mesenteric anastomosis. It is apparent from this study on the rat that most of the preformed anastomoses contribute to the collateral circulation, though the degree of enlargement of each is dependent on a number of factors.

In addition to the larger preformed channels, a localized plexus of fine vessels is evident around the site of ligation. This 'para-aortic' network, well depicted in the illustrations of Porta (1845), receives contributions from the neighbouring parent vessels, and in this series was more prominent when the aorta was ligated proximal to the iliolumbar arteries than when interruption was carried out just proximal to the aortic bifurcation.

The procedure most commonly used consisted of simple ligation by either a single or double thread; section of the aorta between two ligatures caused considerable retraction of the proximal stump so that the branches arising from this segment became angulated, thereby impeding the opening up of the collateral circulation. These findings are in agreement with those of Owings & Hewitt (1942).

The main post-operative sequel noted was a paralysis of the hind-limbs, probably due to a temporary impairment of the blood supply to the lower end of the spinal cord. In the majority of instances this complication resolved after about 3 days. Necrosis of the distal part of the tail was observed in a quarter of the animals undergoing aortic occlusion immediately proximal to the bifurcation. This was no doubt caused by an interference with the blood supply derived from the median caudal artery. All the fatalities which occurred following aortic interruption proximal to the origin of the superior mesenteric artery demonstrated large areas of necrosis of the gut at post-mortem examination.

Finally, it is not justifiable to translate these results obtained in the rat to man, as has been the practice of many of the earlier investigators; one cannot even correlate the findings in the rat and rabbit. In the latter, aortic ligation is always a fatal procedure in spite of the similar vascular arrangements to those in the rat. It is evident, however, that as regards the treatment of aortic aneurysm in man, too often the surgeon has been more concerned with obliterating the aneurysmal dilatation than assessing the state of the collateral vessels, and as the latter may be already taxed to the maximum, the increased burden thrown on a fully developed collateral circulation whose vessels are arterio-sclerotic, may account in part for the invariably fatal termination.

#### SUMMARY AND CONCLUSIONS

1. The origins of the branches of the abdominal aorta have been studied in twenty rats, and ligation experiments have been carried out in thirty-six.
2. The morphology of the collateral circulation was studied in twenty-nine animals surviving the operation.
3. The abdominal aorta can be ligated at all levels distal to the origin of the left renal artery with comparative safety.



4. The macroscopic collateral channels (both visceral and parietal) become noticeably enlarged after a period of 4 days, the degree of their enlargement varying with the site of ligation. The most important of these are the spermatic, mesenteric, ureteric, lumbar and body-wall anastomoses. Additionally, a more localized plexus of fine vessels is present in the region of the ligation site.

5. The main complication following these procedures was a temporary paralysis of the hind extremities of 2–3 days' duration.

6. Attention is drawn to consideration of the potentiality of the collateral circulation when performing similar procedures in man.

I wish to thank Prof. R. G. Harrison for his valuable advice throughout the course of this work. I am also indebted to Messrs L. G. Cooper and C. T. Fitzsimon for their assistance with the radiography and Mr D. Kidd for the diagram. This work has been aided by a grant from the Medical Research Council.

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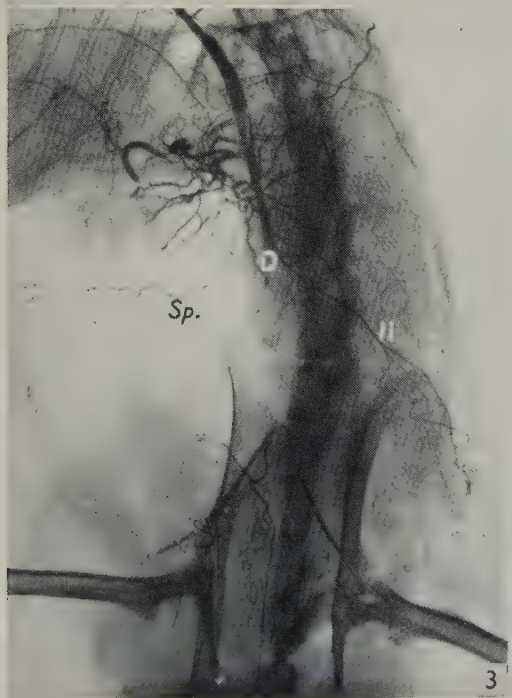
#### EXPLANATION OF PLATES

##### Key to figs.

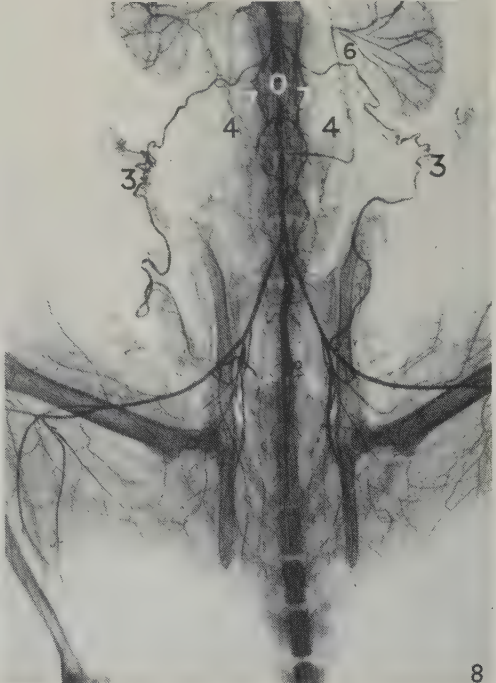
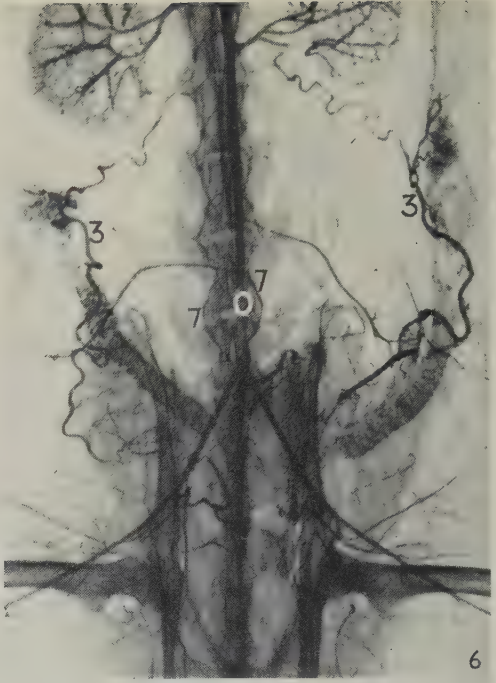
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|--|--|
| 0 Site of occlusion                    | 4 Ureteric anastomosis                         |
| 1 Antero-lateral body-wall anastomosis | 5 Iliolumbar—deep circumflex iliac anastomosis |
| 2 Mesenteric anastomosis               | 6 Iliolumbar—inferior phrenic anastomosis      |
| 3 Spermatic anastomosis                | 7 Lumbar anastomosis                           |
| <i>Ic.</i> Lower intercostal arteries. | <i>Ie.</i> Inferior epigastric artery.         |
| <i>Sc.</i> Subcostal artery.           | <i>Il.</i> Iliolumbar artery.                  |
| <i>L.</i> Lumbar artery.               | <i>Sp.</i> Ovarian (spermatic) artery.         |

##### PLATE 1

- Fig. 1. Arteriograph of control animal—'Micropaque' injection.  $\times 1.5$ .
- Fig. 2. Arteriograph of animal sacrificed within 5 min. of ligation of the aorta proximal to the bifurcation—'Micropaque' injection.  $\times 1.5$ .
- Fig. 3. Arteriograph of animal in which the aorta has been divided proximal to the origin of the inferior mesenteric artery. The animal died within 24 hr. of operation—'Micropaque' injection.  $\times 1.5$ .
- Fig. 4. Arteriograph of animal sacrificed 24 hr. after ligation of the aorta proximal to the bifurcation—'Micropaque' injection.  $\times 1.5$ .









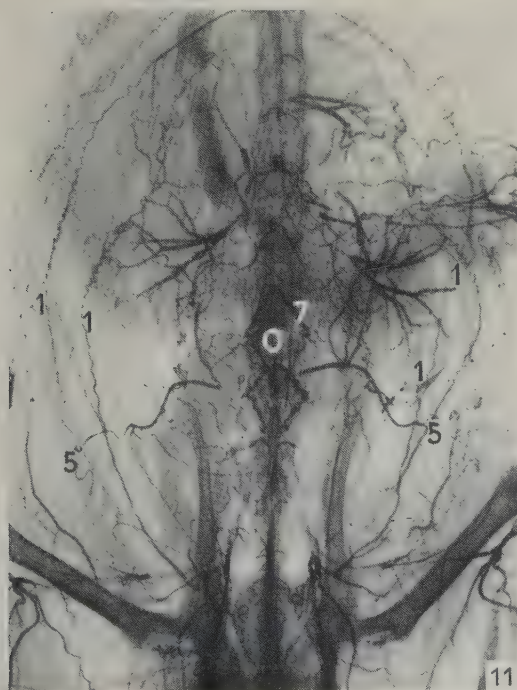
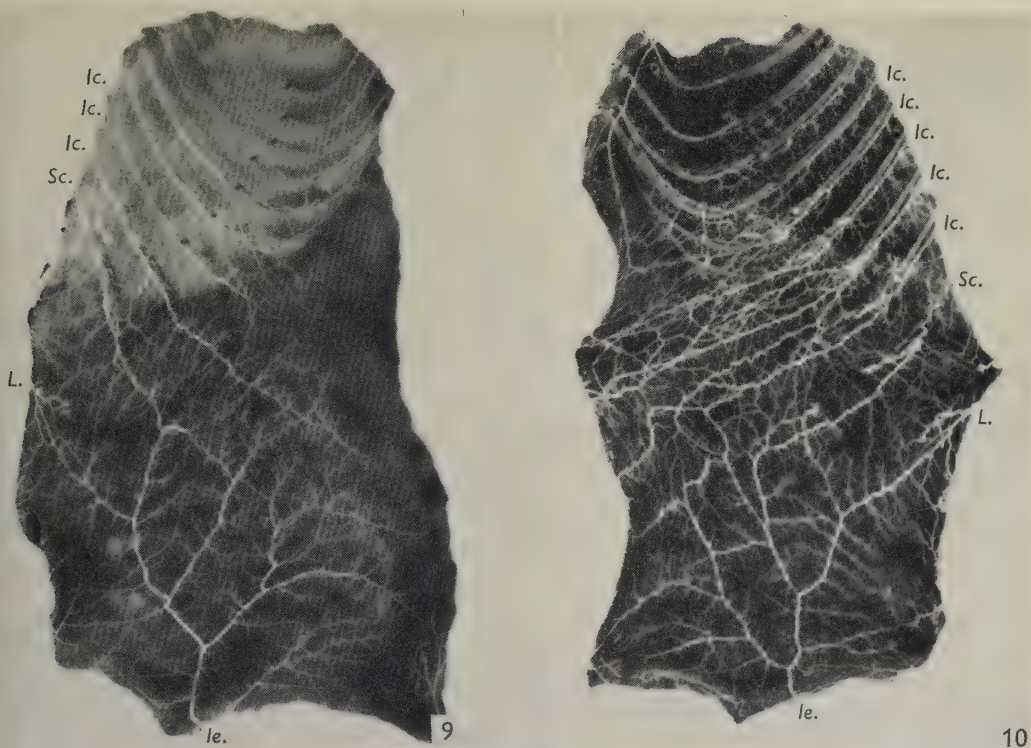




PLATE 2

- Fig. 5. Arteriograph of animal sacrificed 10 days after ligation of aorta proximal to the bifurcation, with the gut retracted—'Micropaque' injection.  $\times 1.5$ .  
Fig. 6. The same specimen as in fig. 5 after removal of the gut.  
Fig. 7. Arteriograph of animal sacrificed 2 months after aortic ligation proximal to the origin of the iliolumbar arteries, with the gut retracted—'Micropaque' injection.  $\times 1.5$ .  
Fig. 8. The same specimen as in fig. 7 after removal of the gut.

PLATE 3

- Fig. 9. Antero-lateral body-wall from control animal (cleared by the Spalteholtz method).  
Fig. 10. Antero-lateral body-wall from an animal sacrificed two months after aortic ligation proximal to the iliolumbar arteries (cleared by Spalteholtz method).  
Fig. 11. Arteriograph of animal sacrificed 3 months after aortic ligation proximal to the origin of the iliolumbar arteries combined with bilateral division of the ovarian (spermatic) arteries. 'Chlorbismol' injection.  $\times 1.5$ .



# BONE GROWTH: A STUDY OF THE GREY-LETHAL AND MICROPHTHALMIC MUTANTS OF THE MOUSE

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## INTRODUCTION

### (a) THE USE OF MUTANT GENES IN DEVELOPMENTAL STUDIES

The use of mutant genes in replacing operative techniques, especially in mammalian experimental embryology, is now a well-established procedure. The method as outlined by Grüneberg (1948*a*) requires the tracing back of the ultimate manifestations of the genes through plausible channels of cause and effect to manifestations ever earlier in their first appearance—the 'pedigree of causes'. In no single analysis of this nature can complete faith be placed in the conclusions obtained, though the postulated pathways may be accepted if there is agreement in the interpretation of several syndromes.

In the present work, however, though the mutant genes are again used to replace alternative operative techniques, they are used in a different way. Only one of the manifold effects of the genes is selected for study—that which affects the growth of the skeleton. The anomalies are first proved to result from the retardation of accretion and from the lack of erosion, and are then translated into a description of the processes of accretion and erosion in skeletal growth.

### (b) AN ACCOUNT OF THE 'EXPERIMENTAL' MICE

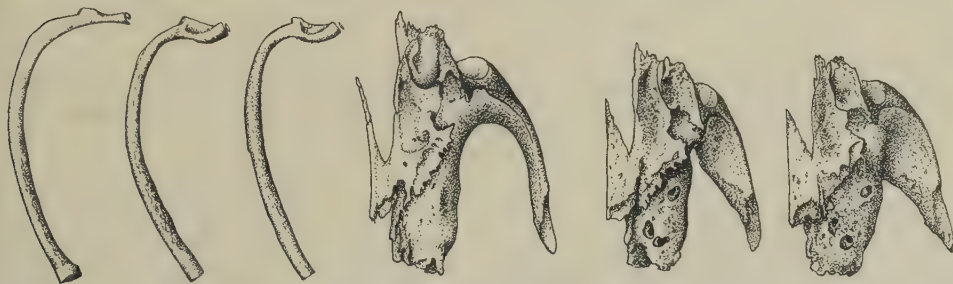
The condition of the skeleton making possible the study of sites of bone erosion and accretion arose twice, by spontaneous mutation, in quite unrelated stocks of mice bred in genetical laboratories.

The skeletal condition was first discovered in the mouse by Grüneberg (1935, 1936, 1937, 1938) and is a distinctive feature of grey mice called grey-lethals (symbol *gl/gl*) which regularly die around weaning. Grüneberg attributed the skeletal anomalies to a 'general arrest of development' and to 'failure of the secondary modelling of the bone surface by absorption'. Skeletal retardation was not unexpected since the mice were much smaller than their normal litter-mates, and at three weeks the degree of ossification of their skeletons was at a stage reached by normal mice when only 10 days old. Failure of erosion was inferred:

- (i) from the small size of intraosseous foramina (which can be enlarged only by erosion),
- (ii) from the fact that the teeth do not erupt, but lie crumpled in their unenlarged sockets,
- (iii) from the persistence of the *spongiosa*, and
- (iv) from the excessive grossness of the zygomatic arch and other bones, and of the growing ends of long bones, which gross formations largely correspond to the sites

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of erosion expected from Kölliker's work (1873). The erosional anomalies are no doubt related to the gigantic osteoclasts of the grey-lethals recorded by Barnicot (1947). Besides causing this extensive pathological syndrome and the grey coat colour, the grey-lethal gene irregularly produces kinked vertebrae near the tip of the tail.



Text-fig. 1. Drawings of a cartilage bone (the seventh rib) and a membrane bone (the maxilla) of normal, microphthalmic and grey-lethal mice respectively; demonstrating the striking similarity of the mutants' skeletons.

An almost identical condition of the skeleton was discovered again by Grüneberg (1948*b*) in mutant mice originally bred by Hertwig (1942*a, b*). The skeletons are so alike it is impossible always to distinguish the microphthalmic skeletons from those of grey-lethals (Text-fig. 1), though they are generally less retarded and erosional failure is apparently not always so complete. At 3 weeks they resemble in the development of their skeletons normal mice of the same stock which are only 14 days old; and the consequences of erosional failure are so variable that some mice live till long after weaning; indeed, one male has survived to sexual maturity and has bred successfully (Hertwig, 1942*b*). Besides their anomalous skeletons and their rudimentary eyes (from which they get their name), the mice are white, their eyes pink, some of their whiskers are kinked near the tips, and, like grey-lethals, their tails too are irregularly kinked.

The heterozygotes (+/*mi*) can be recognized by the variable spotting on the head, belly and tail, singly, or in any combination, and by their reddish eye colour. The heterozygote's tail is more rarely kinked than the homozygote's. It is of interest that in spite of these heterozygous expressions, the present author could detect no heterozygous manifestation of the gene either on the rate of growth of the skeleton or on the inhibition of erosion. This remarkable independence of the pleiotropic effects of the microphthalmic gene was also indicated by selected lines in which the heterozygotes were characterized by quite different expressions of the gene. For example, in one line the heterozygote usually had light eyes but very little spotting; in another, light eyes, a large spot on the forehead but none on the belly; while another had dark eyes and a large head spot. In other words, the gene can be made to manifest well in one direction without necessarily manifesting well in another. The pleiotropy is being investigated by Prof. Hertwig and her associates.

In homozygous grey-lethals and microphthalmics the anomalies attributable to

retardation and to erosional failure are so widespread that it is reasonable to suppose that no bone in the mutant animals escapes the genes' action. Moreover, it seems on first investigation (and subsequent work described here confirms early impressions) that all the regular skeletal anomalies of the mutants can be explained in terms of these anomalies. It was for these reasons that the two stocks of mice appeared to Dr Grüneberg as admirably suited for the study of accretion and erosion in bone; and it was in response to his request that the present author undertook this work.

All the mice required were kindly supplied by Dr Grüneberg. In all, the skeletons of fourteen grey-lethals and twenty microphthalmics were prepared, together with those of at least one normal litter-mate for each mutant animal.

(c) DETAILS OF PREPARING THE SKELETONS FOR ANALYSIS OF THEIR ANOMALIES

Although the anomalies of the mutant mice are likely to become increasingly pronounced in older material, rendering the analysis of the differences more easy, certain and subtle, the optimum age at which to prepare the skeletons was found to be 3 weeks, since only a small proportion of the mutant animals could be expected to live and grow much beyond this age. This was associated with a disadvantage, however, that some of the bones, for example, the Tympanohyoidea (Johnson, 1933), would not even have begun to ossify.

The larger bones (skull, lower jaw, girdles and larger long bones of the limbs) were prepared by maceration in boiling water. It was found quite easy to remove the flesh with forceps, scalpel and scissors after only 20 min. boiling. There was little danger of disarticulating the epiphyses or compound bones except when eviscerated animals had been stored in the refrigerator for some days. The brain was removed by jets of water from fine pipettes, the dura with the aid of forceps.

Isolated skull bones were prepared by papaine digestion (Luther, 1949), as even dilute solutions of potassium hydroxide were found to damage the bones. Whether macerated in boiling water or by papaine, the bones were then defatted in acetone and bleached with hydrogen peroxide.

For the remaining bones which were small or only partially ossified, neither of these techniques was found suitable. These bones were made available for examination by staining whole mice (skinned and eviscerated) with alizarin, and then clearing and differentiating (the method used was a modification of Johnson (1933)).

For examination, macerated bones were placed on plasticine so that they could be supported in unstable positions. Bones from transparencies were cut from the rest of the skeleton and placed in a glycerol bath. This consisted of a wax square made in three depth sizes, filled to overflowing and placed between a glass plate and microscopic slide. Distortion was thereby reduced to a minimum, and by moving the square on the glass disc, or the slide on the square, the bone could be orientated and held in almost any desired position.

The bones were then drawn, the extreme variation in their opacity rendering them quite unsuitable as photographic subjects. The outline was made accurately by camera lucida attachment to one eyepiece of a low-powered binocular microscope



after which shading was added free-hand. Magnification of the original drawings was usually  $\times 9\frac{1}{2}$ , but this has been reduced to  $\times 6\frac{1}{2}$  for publication. Moreover, as the similarity between grey-lethals and microphthalmics is very strong, only the former have been reproduced in the appended plates.

Diagrams are free-hand interpretations of the original drawings or of superimposed tracings of them. The arrows indicate directions of growth.

#### (d) TECHNIQUES OF STUDYING BONE GROWTH

The techniques of studying bone growth are many. The earliest techniques in which holes were bored and metallic marks were fixed near the ends of long bones (Stephen Hales, 1727) are of little more than historic interest, being the first to show that growth of long bones is localized terminally.

A more subtle technique was developed by John Hunter (1835). This was the feeding of madder which stains bone laid down during the period of madder-feeding a pinkish colour. Bone laid down subsequently is recognized by being white against the pink background. This method, which is applicable to the study of surface accretion in both long and flat bones, was used also by Flourens (1840), but the works of both these early writers are almost inaccessible. The latest workers in this field (Brash, 1934; Payton, 1932, 1933) have not described the growth of an entire skeleton between them. While the madder technique is limited to those animals which will eat it, a parallel technique of much greater scope has been developed by Hoffman & Schour (1940*a*). These workers replaced madder-feeding with the injection of Alizarin red S, a stain related to madder and one of the anthraquinone group. New bone is recognizable within 3 hr. of the injection and retains its colour for at least 4 months. Recognition of the sites of bone accretion at successive injections would be facilitated if a different anthraquinone stain, having a contrasting staining reaction (Brash, 1939), were used for each injection.

A quite different technique has been evolved by Harris (1933). He noticed that bone formed during severe illness is extremely dense and can be recognized in radiographs many years afterwards. The extent to which these 'lines of arrested growth' are capped by later formed bone therefore affords a precise measurement of the extent of accretion. The dense lines are always formed adjacent to the conjugation cartilages and periosteum and therefore identify these as the sites of bone accretion. Boerema (1942) has made similar use of zones of dense bone formed as a result of seasonal consumption of phosphorus drugs. He met with special difficulty, however, in applying this method to the study of surface accretion in the vault bones of the skull.

Yet another technique is the use of radiophosphorus (Leblond, Wilkinson, Bélanger & Robichon, 1950). This substance is incorporated by newly formed bone and autoradiographs can be obtained from sectioned material. This technique is especially useful in studying histological aspects of bone accretion.

The rates of growth at the ends of long bones has also been estimated by counting the number of cells in the ranks of the conjugation cartilages (Harris, 1933).

In none of these studies was it possible to observe directly the extent of bone erosion, though several authors have inferred erosion from their observations on

accretion. Kölliker alone (1873) attempted a simultaneous study of accretion and erosion, but his accounts of the two aspects of bone growth did not relate to the same species and were treated in different sections of his book. Kölliker described the *sites* of erosion from the occurrence of Howship's foveolae (formed by the osteoclasts) or of the osteoclasts themselves in thick tangential sections of softened bone. His method, however, was unable to provide information on the *rates* of erosion, since it seems that osteoclasts vary tremendously in their activity, and mere numbers afford no guide to the intensity of erosion. Thus not only did he observe osteoclasts before the formation of bone, but he also figures bone surfaces for which it is difficult to imagine osteoclasts more crowded together, and others which, although pot-marked all over with Howship's foveolae, present hardly any osteoclasts (compare his figs. 3, 6 and 9 with his fig. 5). Moreover, Ruth (1937) reports that he and Kawata (1924) observed comparable amounts of erosion in the pelvic symphysis of the guinea-pig during pregnancy but very different numbers of osteoclasts. Lastly, Barnicot (1947) described a massed migration of osteoclasts across the parietal bone of the mouse, although it is unlikely that there was any change in the sites of erosion.

The grey-lethal-microphthalmia technique used here has one major advantage over all the preceding methods—for it provides direct information on the rates of erosion. It displays the sum total of accretion and erosion over a long period, and unlike Kölliker's method, never depicts erosion which is only temporary and occurs at the time of death. Owing to the sublethal action of the mutant genes the technique is necessarily confined to very young animals, but this has resulted in the provision of some novel contributions to our concepts of bone growth. The method is also applicable to almost every bone in the skeleton.

Such an attractive method is not, of course, without its handicaps. In the first place it is not possible, as with the other techniques, to use a bone as its own 'control'; in fact, it is necessary to make comparisons between drawings of two bones. Thus error can be introduced into the analysis through ascribing differences which may occur between any two bones to the actions of the segregating genes. In the second place, the condition which makes the study possible is limited as a regular occurrence to the mouse and rat. In the rat the condition is inherited as a simple recessive (Bhaskar, Weinmann, Schour & Greep, 1950) and is only temporary. Recovery begins at about 30 days, and the skeleton is often completely normal by 150 days. Nevertheless, those authors have used the condition in a microscopic study of the growth processes of the tibia and humerus of the rat. It may be noted in passing that the Sirenia (Dugong and Manatee) exhibit a skeletal condition which may be regarded as a physiological grey-lethal. However, although the spongiosa persists, it seems that erosion of the external surfaces of the bones is in normal quantities. Sporadic cases of the condition have occurred in the rabbit and in man where it is known as osteopetrosis. Albers-Schönberg (1907) gave the first account, and there have been subsequent reports by Elliot Smith & Wood Jones (1910), by Suk (1929) and by Lightwood & Williams (1940). Ingalls & Grossberg (1932) have described it in a 'unique' pair of femora but failed to see the significance of the anomaly and did not mention whether other parts of the skeleton were affected.

(e) THE ANATOMICAL ATLAS

The present method of recognizing the sites of accretion and erosion necessarily entailed comparing camera lucida drawings of normal and mutant bones, and the drawings of the normal bones have been extensively labelled to serve as an atlas of the mouse skeleton (see Pls. 1-20). Although some precedent has been set by Greene (1935) in her *Anatomy of the Rat*, her account of the skeleton is inadequate for many anatomical purposes.

In this atlas the author has used the latinized veterinary nomenclature of Ellenberger & Baum (1926) which is preferable to the related BNA nomenclature (Jamieson, 1916), since it is adapted to four-footed animals. But where example has been lacking in Ellenberger & Baum the author has found it necessary to use BNA terms and even to invent new ones (recognizable in the text by the initials N.B. in parentheses).

The latinized nomenclatures may be a source of difficulty for the English reader but have the advantages of being the most complete applicable nomenclature and therefore the least likely to require the addition of new terms, and also of being the most standardized and universally recognized.

(f) THE PROBLEM OF SUPERFICIAL AND INTERSTITIAL GROWTH OF BONE

Unlike the madder technique, which reveals only the superficial component of bone growth and is independent of the occurrence of interstitial growth, the method used in the present work is based on the assumption that bone growth is entirely a surface phenomenon; and the conclusions presented in the subsequent pages would be quite invalidated if any interstitial growth of bone occurred. Further, unlike Harris's or Boerema's techniques where the lines of dense bone can be shown in successive radiographs to have remained at their original distances apart, the present method is incapable of providing complete proof of the absence of interstitial growth. It is therefore necessary carefully to examine these alternative theories as to the method of bone growth.

It is unnecessary to review in detail the case for the superficial growth of bone. Each of the diverse techniques described in an earlier section of this paper provides independent evidence for the existence of superficial bone growth. Further evidence is supplied by a series of embryological experiments performed by Lacroix (1942-3, 1946*b*) which involved the transplantation of pieces of conjugation cartilage and periosteum and proved that both are centres of bone production. The existence of bone erosion is itself evidence for surface accretion, since it is impossible for the complex shapes of most bones to be maintained merely by differential rates of accretion. The widespread occurrence of bone erosion recorded by Kölliker's classic work (1873) is proof of the widespread nature of surface accretion. Lastly, the skeletal deformities whose detailed investigation forms the bulk of this account can be explained quite simply on the assumption that surface accretion is retarded and that no erosion takes place. On the other hand, anyone believing solely in the interstitial growth of bone would find it extraordinarily difficult to explain the anomalies.

In spite of the weight of evidence for the superficial nature of bone growth, the



concept has met with recurrent resistance from many osteologists. Their objection seems to be largely philosophical, regarding 'meristematic' growth as the sole property of the botanical world. Yet 'meristems' are found in the skin and in the ovary, and the bones of the limbs and girdles of the chick are delineated from the meristematic apical cap of the limb-bud (Saunders, 1948).

Compared with the experimental evidence for superficial growth that for interstitial growth is inadequate. None was available until 1929 when Kornew claimed to have demonstrated it. He surrounded the ulnar and fibular metaphyses of rabbits with metallic rings (i.e. at each end of the bone on the highly concave part near the conjugation cartilages). The rings moved farther apart as the bones grew in length, although they were placed behind the sites which are usually regarded as the regions where superficial accretion takes place. However, unless the rings were deeply embedded in the bone substance they were more likely to trace the growth of the periosteal membrane than of the bone itself, and it seems highly probable that Kornew's experiments merely demonstrated the interstitial growth of the periosteum!

Bisgard & Musslemen (1940) made unilateral bone grafts between exposed cancellous surfaces of the vertebral centra of month-old goats. They succeeded in getting bony continuity between four contiguous vertebrae in two cases and between two vertebrae in one other case. Even 10 months after the operation the ankylosed regions in no case showed any sign of bending, for the grafts themselves had grown. Although the authors had just demonstrated that the centra (corpora vertebrae) grow at equal rates at their two ends by ossification of the conjugation cartilages, they nevertheless concluded that the grafts had grown interstitially. But Lacroix (1946*a*) has suggested that the grafts underwent a series of profound histological changes, which do not occur in normal growth, involving decalcification followed by redeposition of the bone, this time at new surfaces.

Boerema (1942), who was using dense layers of bone produced by seasonal drugging with phosphorus compounds to show up the pattern of growth of the human skeleton, concluded that interstitial growth of the vault bones of the skull was not excluded, since these bones, in contrast to those of the limbs and girdles, showed no osteosclerotic lines. However, if their growth were superficial, there would have to be so much bone erosion and replacement that it is most probable that the original osteosclerotic lines would not sufficiently survive the passage of years as to be visible in radiographs.

Lastly, as late as 1946, Leveuf used in evidence of interstitial growth failure of melanic exostoses (bony protuberances from the sides of the diaphyses) to become more distant from the growth (conjugation) cartilages. However, Lacroix considers this also inconclusive, because nowhere does Leveuf state that the exostoses are not remodelled, and that growth at the diseased end has not ceased. Either remodelling or cessation of growth at the diseased end could account for the situation in spite of superficial growth.

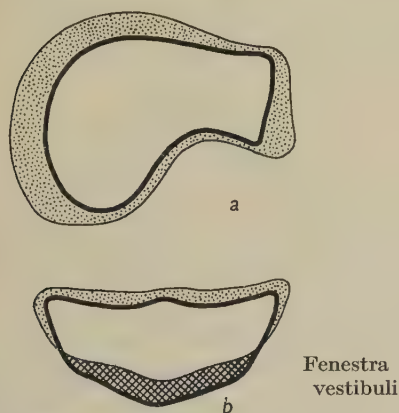
It is essential to make a clear distinction between interstitial *accretion* and *erosion* (erroneously believed to be directly involved in bone growth) and interstitial changes of *deposition* and *decalcification*. Both the latter are regular, but only accessory features of superficial bone growth, being necessary components of the

histological changes from spiculate (endochondral) and from dense (periosteal) bone to the canalated (endosteal) bone of Haversian systems. While these processes confer on bone a certain plasticity of structure, the changes occur within the rigid framework of the outer layers of bone and cannot be directly involved in its growth. Interstitial decalcification results from osteolytic action of the cells contained within the spicules (Lacroix, 1942-3).

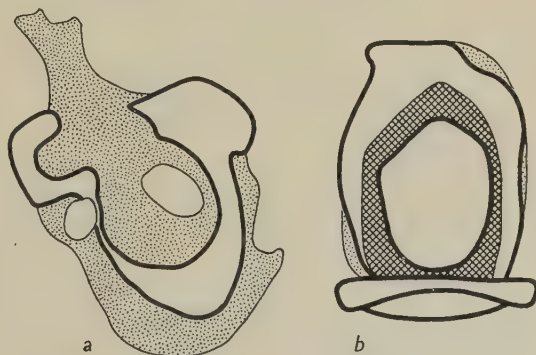
It can therefore be concluded that the present technique rests securely on the establishment of superficial accretion and erosion as general phenomena and on the banishment of interstitial growth to, at most, only rare expression.

(g) SOME BASIC PATTERNS OF BONE GROWTH

Although in bone growth we are concerned with the two opposed forces of accretion and erosion, they are, in fact, two intimately related and complementary processes, and the need is felt for substituting previous authors' static terms of 'site of accretion' and 'site of erosion' by a terminology which attempts to conjure






Text-fig. 2. (a) External accretion in the caput humeri. (b) External erosion in the os interparietale.



Text-fig. 3. (a) Internal accretion in the pars petrosa between birth and 3 days. (b) Internal erosion in the stapes.

Key. In figures 2-48 the growth processes differentiating older or normal bones (thin outlines) from younger or mutant bones (heavy outlines) are shown as follows:

Bone deposited by accretion  Bone removed by erosion   
 Bone which is first deposited and then eroded 

Bone substance common to old and young, or normal and mutant, is left unshaded.

Thus outlines of older or normal bones enclose unshaded and stippled areas; and outlines of younger or mutant bones enclose unshaded and cross-hatched areas.

Arrows indicate directions of growth revealed by the mutants, and are lines of reference in superimposing the outlines of normal on mutant bones.

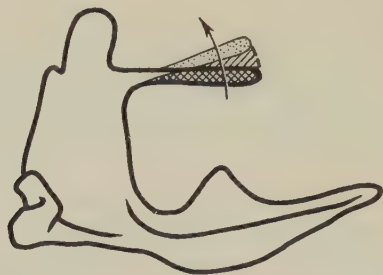
Only those processes visible in optical section are shown.

up a picture of a co-ordinated system of movement in place of a picture of haphazard scattering of static sites of accretion and erosion. In the remaining part of this section eight theoretical interrelations of the sites of accretion and erosion are described, each of which has actually been observed in practice. Each is given

a descriptive name so that it may be remembered the more easily, as it will be referred to many times in subsequent sections.

First let us consider those movements which result from only one growth process. Accretion and erosion, the two processes concerned in bone growth, can each have two situations in relation to the bone (viz. on the external and internal surfaces). Hence there are four basic movements in growth. These are:

(i) *External accretion* (Text-fig. 2a). External accretion is characteristic of the head of the humerus (caput humeri). New bone is deposited at different rates over the surface of the existing bone. Alone, it suffices to maintain and to develop the bone's shape.



Text-fig. 4. Unilateral growth of the manubrium mallei.



Text-fig. 5. Diaphyseal growth.

(ii) *External erosion*. External erosion does not in itself result in growth and is probably always associated with some accretion. In the interparietale (Text-fig. 2b) it is the dominant process, so that the bone actually becomes increasingly shorter after 8 days from birth.

(iii) *Internal accretion* (Text-fig. 3a). In internal accretion, bone is deposited on the inner surface of the hollow bone, causing closure of the cavity, canal or foramen enclosed by the bone. It is of transitory nature. The fenestra vestibuli of the perioticum is an example of it, having at birth three times its final diameter (the perioticum grows also by external accretion).

(iv) *Internal erosion*. Intraosseous foramina are enlarged by internal erosion. Text-fig. 3b shows how the foramen stapedis is enlarged by internal erosion.

These four basic patterns may be combined in pairs to form four other known growth patterns of varying complexity, occurring sufficiently frequently to be described.

(v) *Unilateral growth*. In unilateral growth the first and second patterns are combined such that accretion is restricted to one side of the bone with erosion of the opposite and oldest side of the bone (Text-fig. 4).

In this way the manubrium mallei is bent away from the processus longus. In this case, unilateral growth is inferred from the difference between normal and mutant mice in the angle between the two processes. The thickened process theoretically expected to arise in the mutants with this growth pattern has not been formed. This additional anomaly—complete failure of accretion—is special justification for regarding this pattern sometimes as a unit process, and not as a combination of two more or less independent activities.

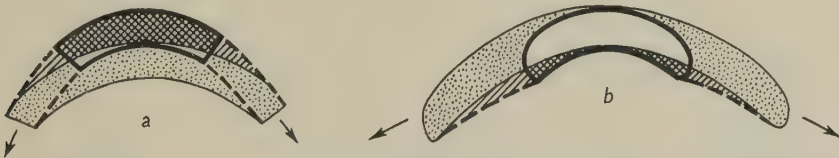


On the other hand, the two processes (external accretion and external erosion) are independent of one another in the processus zygomaticus of the maxilla. Here, in the mutants, failure of erosion on the medial side does not totally inhibit accretion to the lateral side and the process becomes exceptionally thick (Pl. 13).

*Reversed unilateral growth.* When a foramen migrates it is said to do so by the 'reversed unilateral' pattern of growth because *internal erosion* leads the way and *internal accretion* follows behind—a double reversal of the normal 'unilateral' process in which the patterns are external and in which the sites of accretion and erosion are interchanged.

(vi) *Diaphyseal pattern.* Both accretion and erosion are external as in unilateral growth, but, unlike it, the erosional component tends to act at right angles to, and not opposite, the site of accretion; so that the newest, and not the oldest bone, is eroded (Text-fig. 5). The pattern is typical of the rapidly growing ends of all long bones.

The last two patterns to be considered are concerned with the growth of curved bones which, unlike the stapes, are not complete rings or cylinders; and they are alternative to the joint activities of external accretion with internal erosion already described for the stapes. In both, growth is primarily by peripheral accretion, but they differ radically in their secondary, remodelling processes.



Text-fig. 6. (a) Centripetal growth: ultra-diagram of an arcus vertebrae.  
(b) Centripetal growth: ultra-diagram of the os parietale.

(vii) *Centripetal growth.* In many arcus vertebrae (neural arches), before their fusion with the centra and with each other, the remodelling process takes the form of accretion to the internal, concave surface, while erosion is external. The name centripetal growth has been thought appropriate to this pattern, since there is a steepening gradient in the extent of remodelling from the ends of the arch to its centre (Text-fig. 6a). (Although an arched bone has been taken as our example, the pattern is equally applicable to a bone which forms part of the shell of a sphere, in which case Text-fig. 6a represents a diametrical section across the bone.)

(viii) *Centrifugal growth.* In this case the secondary remodelling processes are most intense peripherally and migrate outwards as the bone grows. Also in contrast to the last pattern, accretion is external while erosion is internal (Text-fig. 6b). Centrifugal growth plays an important part in the growth of the parietale and frontale.

It should be made quite clear that these eight patterns are not hard and fast classifications of types of bone growth, but merely classifications of convenience, helping to make the description of growth vivid. The choice of pattern to describe a process is often arbitrary. For example: enlargement of the incisura lacrimalis of the maxilla (p. 237) can be regarded as resulting:

- (1) from 'internal erosion' (of the borders of the incisura),
- (2) from 'unilateral erosion' (when the site of erosion is regarded as on the posterior

wall of the lamina infraorbitalis and account is taken of the accretion to its anterior margin), or

(3) from 'diaphyseal erosion' (when account is taken of accretion to the upper border of this lamina).

Several similar instances occur when the bones are similarly complex in shape, but the author does not consider that these situations will cause confusion.

There are of course many other combinations and interactions, but it is felt that these are too complicated and too rare to be usefully described here.

#### (h) THE METHOD OF ANALYSIS

The method of interpreting the anomalies of the mutant mice in terms of accretion and erosion consists first in recognizing in these anomalies the characteristic malformations resulting from any of the above basic patterns of growth. In some instances those discoveries may serve to display the direction in which particular features of the bone are growing, and if there are sufficient of such lines of reference, the outlines of the mutant and normal bones may then be superimposed so that all growing points coincide along the same lines. These lines of reference are indicated in the text-figures by arrows. At other times, it is only possible to arrange the superimposition so that none of the known features of the bone's growth is excluded. At all times the superimposition is only regarded as correct when there is total agreement as to the sites of accretion and erosion whichever mutant (grey-lethal or microphthalmic) is used in the analysis. It is noteworthy that substantial differences in the form of the two mutant bones such as occur for the pubis (Text-fig. 7) in no way affect the conclusion as to the manner of *normal* bone growth. This implies that variations in the form of identical bones occur within the same general pattern of growth and are not due to differences in the patterns themselves. (The causes for these variations are treated more fully in the Discussion.)

When the superimposition is made, and for as many angles of viewing as seems desirable, all the growth processes become detectable, and these are then described in terms of the patterns of growth detailed above.

Only in one instance has there been an unforeseen and serious but unavoidable discrepancy in the results of the analysis based first on the grey-lethal bone and then on the microphthalmic; but in this case, independent evidence was later obtained which demonstrated that a change of growth pattern did occur between 10 and 14 days, which are the developmental ages of the two different mutant animals used in the analysis.



Text-fig. 7. Outlines of the pubic bones of a microphthalmic mouse (left) and of a grey-lethal (right). These substantial differences in shape do not affect the results of analysis as they occur within the same and normal pattern of growth. They are due to differences in the time and rate of ossification.

## GROWTH OF MOUSE BONES

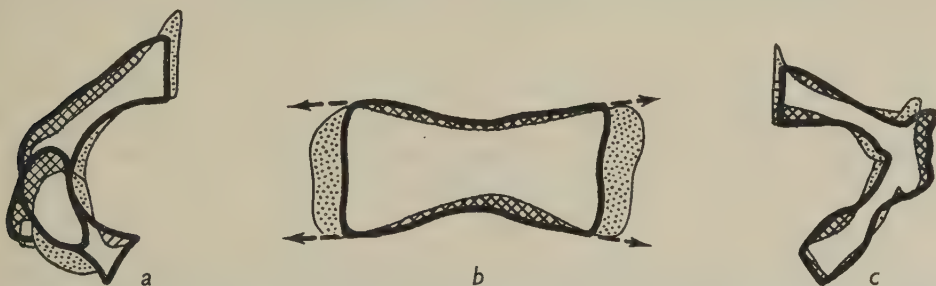
### (a) VERTEBRAL COLUMN (Pls. 1-5)

During their early development the vertebrae change from three-piece to unit construction—through the fusion of the two arcus vertebrae with the corpus. The transition occurs between 1 day after birth (for the caudal vertebrae) and 14 days (for the cervical vertebrae), so that each vertebra presents a compound picture of two entirely different and successive patterns of growth which cannot be distinguished in any individual case. But thanks to their repetitive nature of growth and a postulated repetition in their manner of growth, it is possible if not entirely legitimate to sort out the information gathered throughout the vertebral column to reveal the two patterns of growth. The following account is exemplified only by those vertebrae which were drawn, they being chosen to demonstrate the variation in vertebral form. (The names of bones in brackets give the origin of the evidence for the statement which precedes it.)

#### *The early phase of growth*

##### (i) *Arcus vertebrae* (Text-fig. 8a, c)

During the early period of growth the arcus vertebrae grows 'centripetally' usually with 'diaphyseal' growth at the initially wide synchondroses at both the interneural and neuro-central sutures (atlas, epistropheus (axis), sixth cervical,



Text-fig. 8. Centripetal growth of the vertebral arches of the epistropheus (left) and seventh thoracic vertebrae (right). The middle figure shows diaphyseal growth of the vertebral body of a caudal vertebra.

and second and seventh thoracic for the former; and second and seventh thoracic for the latter). While the direction of growth of these 'diaphyseal' components varies considerably between vertebrae (contrast the epistropheus and second thoracic), it is general for this component to contribute more to the breadth than to the height of the bone; a tendency which is corrected by the 'unilateral' component of the 'centripetal' pattern.

The failure of this 'unilateral' accretion results in excessive breadth of the foramen vertebrae in the mutant atlas, epistropheus, seventh thoracic and lumbar vertebrae. And the failure of the corresponding 'unilateral erosion' is seen in the excessive breadth of the arcus in the epistropheus and second and seventh thoracic vertebrae.

The foramina transversaria move inwards by 'reversed unilateral' growth. The processus transversi grow at their bases by *erosion* of the surrounding region of the arcus with less rapid *erosion* of their tips. The failure of the latter causes the measure-



ment across the vertebrae between the tips of the processus sometimes to exceed those for corresponding normals.

The processus spinosi ossify late. That of the second thoracic vertebra\* does not begin to ossify until the second phase of vertebral growth. On the other hand the spine of the epistropheus begins to form while the bony arcus are still separated by cartilage. The latter spine develops by dorsal extension of the terminal growth zone (Lacroix's 'ossification ring') and, at this stage, there is 'diaphyseal erosion' of the more lateral parts of its base.

The method of analytical superimposition cannot be applied to the study of the growth of the arcus in the antero-posterior axis, for slight differences in the angles from which the bones are viewed can cause large differences in the outline drawings. The observations on the growth are therefore restricted to those limited portions of the bones' circumference which can supply independent information.

Anterior accretion occurs in the atlas in the region of the foramen vertebrale laterale. This foramen is formed by the flow of bone around it. In 7-day-old mice it is still open; and in the much retarded grey-lethal closure has just taken place and the foramen is very near the anterior border. But in the less retarded microphthalmic, failure of 'reversed unilateral growth' by which it normally migrates forward, has left it too far from the anterior edge.

In a similar way, the foramen transversarium of the epistropheus indicates that there is posterior accretion in this region. The posterior border is also the more important site of accretion in the region of the foramen alare ventrale of the atlas. This foramen is formed early in the ossification of the atlas, and is a mere pinprick in the mutants too far from the posterior border.

The other vertebrae afford no direct clues as to the surfaces to which accretion occurs. However, since the corpora (*vide infra*) grow at both ends, it is improbable that the arcus vertebrae differ widely in this respect.

#### (ii) *The corpus* (Text-fig. 8b)

The corpora of the more posterior caudal vertebrae and of the second, third and fourth sacral vertebrae grow in the typical 'diaphyseal' manner at about equal rates at each end. 'Diaphyseal' erosion, however, is limited to the sides and ventral surfaces. In all other vertebrae the waist of the corpus is obliterated by the replacement of 'diaphyseal erosion' by 'external accretion'—thus maintaining their typically rectangular shape.

#### (iii) *The arcus haemales (chevron bones)*

The ragged appearance inside the V of the mutant arcus haemales is certain indication that this is a site of erosion. In consequence there must be accretion to the outside of the V. It is possible that the arcus haemales also grow by terminal accretion to the dorsal ends of their limbs.

### *The second phase of vertebral growth*

The processes so far considered in relation to the diametric growth of the vertebrae depend on their separation, apart from the arcus haemales, each into

\* This spine is absent in the grey-lethal mouse figured in Pl. 2. Grüneberg (1950) has noticed that this spine is absent in about half the animals in the stock.

three more or less independently growing pieces—the two arcus vertebrae and the single centrum. When fusion occurs between these pieces, growth is by no means completed, and profound changes in the patterns of growth are induced. The arcus now grows upwards and outwards by ‘external accretion’ while ‘internal erosion’ (caudal vertebrae and atlas) enlarges the foramen vertebrale—a kind of ‘unilateral growth’ on a curved surface. The processus articulares and transversi also grow upwards but by a ‘unilateral’ component behind the ‘diaphyseal’ growth by which they now increase in length. The spine of the epistropheus no longer grows by an extension of the ‘diaphyseal’ growth of the arches but merely by ‘external accretion’ to its tip. It is not until the second phase that the processus spinosus of the second thoracic vertebrae develops, and here, too, it is by ‘external accretion’. The corpora continue to grow in length by ‘diaphyseal’ growth, and their neural surfaces are still not eroded. Thus their growth does not contribute to increasing the diameter of the foramen vertebrale.

#### (b) THE STERNUM

The sternum has three regions, together composed of seven segments (Pl. 5). The most anterior region is represented by the manubrium sterni which articulates with the clavícula and the first cartilago costalis. Then follows the corpus, constituted by a series of four small sternebrae which are more or less alike in their form and growth. The remaining costal cartilages articulate with them. The processus xiphoideus comprises the last region; it supports the cartilago xiphoidea.

##### (i) *The manubrium sterni* (Text-fig. 9a)

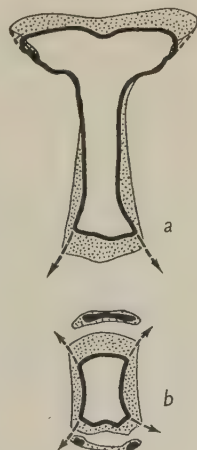
The manubrium sterni of the mouse has a distinct ‘capitulum’ (N.B.) and an elongated ‘corpus’ (N.B.). The capitulum articulates both with the clavicaulae and with the first cartilagine costales; the corpus carries a median longitudinal ventral crest. Growth is ‘diaphyseal’ anteriorly and posteriorly; ‘diaphyseal erosion’ of the ventro-lateral side of the corpus shapes the crest.

The grey-lethal mice do not exhibit the lack of ‘diaphyseal erosion’ in the capitulum, because at their stage of development (10 days) they have only just completed the exceedingly rapid ossification of the perfect cartilaginous model of the capitulum whose onset is deferred until the eighth day after birth. Erosional anomalies are, however, visible in the microphthalmic mice.

##### (ii) *The second to fifth sternebrae*

Growth of these sternebrae (Text-fig. 9b) is similar to that of the presacral corpora vertebrae—by ‘external accretion’ from an ever widening conjugation cartilage, while circumferential ‘external accretion’ fills out the waist into rectangular form.

The paired origin of the sternebrae is reflected in the paired ossification centres which form in the epiphyses around the eighth day. For the moment, at least, epiphyseal growth is by ‘external accretion’ alone.



Text-fig. 9. (a) The manner of growth of the manubrium sterni, and (b) of a small sternebra.

(iii) *The processus xiphoideus*

From the broad, anterior, ossified portion of the processus at birth, ossification spreads posteriorly by 'external accretion' over an ever decreasing surface up to the sixth or seventh day. But subsequently, the posterior extremity widens and 'diaphyseal growth' with erosion especially of the dorsal surface becomes apparent.

## (c) THE RIBS (COSTAE) (Pl. 5)

The growth of the seventh rib is described first as being the most typical; those of the first and twelfth follow as showing the extent of variation in growth. (The thirteenth rib in these stocks is uncommon, and often only unilaterally represented or asymmetrically foreshortened.)

(i) *The seventh rib* (Text-fig. 10a)

Distal growth is about seven times as important as proximal growth. The original curvature of the bone, as seen in the mutants, is due to graded rates of terminal accretion between the lateral and medial surfaces but is highly modified by three zones of 'unilateral' movement. Of these, two (one for the collum; the other extending practically throughout the whole length of the corpus as far as the distal 'ossification ring') are directed laterally, while the third (between the other two) is directed medially. Together, these three 'unilateral processes' serve to accentuate the curvature of the rib and so to enlarge the thorax.

(ii) *The first rib* (Text-fig. 10b)

The terminal rates of growth are probably much more equal than for the seventh rib; and while erosion plays an insignificant role, the accretional components of the 'unilateral' movements persist.

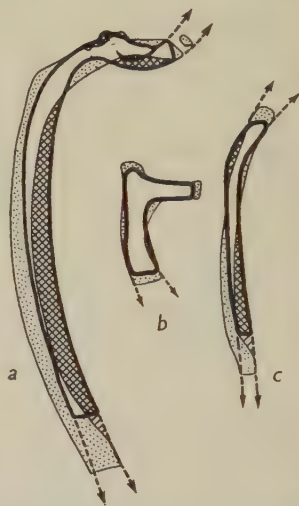
(iii) *The twelfth rib* (Text-fig. 10c)

In spite of its very different shape, the twelfth rib grows in the same manner as the seventh. Analysis of this rib's antero-posterior growth was made possible by the slightness of its lateral curvature which allowed it to be held in the glycerol bath for viewing from the medial aspect. Analysis indicated expansion of the middle of the corpus by accretion to both edges—a feature probably characteristic of rib growth.

## (d) THE SKULL (Pls. 6–13)

Although the skull is a compound bone, so that the growth processes of the individual bones must be neatly interrelated, it is unnecessary to give any general account of its growth.

It may, however, be remarked that unlike the vertebrae, sternebrae and ribs, no two bones of the skull (unless paired left and right) grow in a similar manner. When of complicated form, e.g. maxilla, the concept of a 'punctum fixum' is



Text-fig. 10. The growth of the seventh (a), first (b) and twelfth ribs (c). Note the importance of the unilateral pattern.

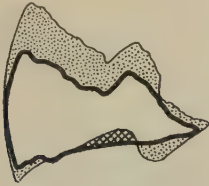


invalidated unless it is extended to encompass a position somewhere in the spaces between the bone's several processes and laminae. The squama occipitalis, sphenoidale orale and lacrimale may be cited for exemplifying the dependence of some bones on the normal growth of their neighbours for their own normal growth. Lastly, the occipitale and tympanicum may be cited for displaying similar age changes in their growth patterns to those encountered in the vertebrae.

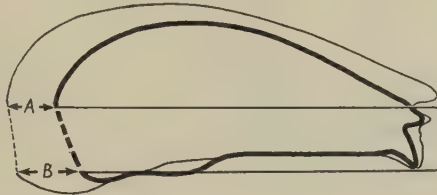
(i) *Occipitale: pars basilaris* (Pl. 7)

'External accretion' occurs at all borders but especially at the synchondroses with the partes laterales and with the sphenoidale aborale. Erosion occurs only on the inferior surface, where, on either side of the mid-line, just in front of the posterior margin, it brings out into relief part of the tuberculum pharyngeum.

*Pars lateralis* (Pls. 7, 8 and 9). There is 'external accretion' at the synchondrosis with the pars basilaris and along the margin of the foramen magnum. In pace with this growth, the canalis hypoglossi migrates medially by 'reversed unilateral growth'. The material is unsuited for further analysis.



Text-fig. 11. Growth of the squama occipitalis.



Text-fig. 12. The foramen magnum. Differential rates of growth of the floor and roof of the skull cause the foramen magnum to assume a more upright position.

*Squama occipitalis* (Pls. 6 and 8 and Text-fig. 11). In both its horizontal and vertical planes growth is 'centrifugal'. Failure of the proper co-ordination of the growth processes of adjacent bones in the mutants results in the corrugation of the anterior growing region by the strains exerted on it by the backwardly growing squamosum; and the overriding of the squama by the interparietale owing to erosional failure in the latter.

*Foramen magnum.* The growth processes intrinsic to the occipital bones do not solely account for the gradual assumption of a more vertical position by the foramen magnum. Part of this new orientation is due to the different rates of growth which affect the roofing bones as a whole compared with the basal bones as a whole (Text-fig. 12).

The squama occipitalis fuses with the partes laterales at about 17 days, and the occipitale becomes a single bone at about 20 days. While the present material is too young to demonstrate the new growth pattern there is little doubt that 'centrifugal' growth of the upper margin of the squama contributes most to the height of the bone, while 'centrifugal' growth in the horizontal plane, and 'external accretion' at the synchondrosis speno-occipitalis, contribute to its growth in length.

(ii) *Sphenoidale aborale* (Pls. 7-9 and Text-fig. 13a, b)

The corpus grows at probably equal rates backwards and forwards by 'diaphyseal' growth in which the erosional component is strictly limited. The alae temporales grow outwards and upwards by 'external accretion' to their lateral and posterior margins, while their roots are more widely separated by a graded 'unilateral' movement which falls away towards the upper growing margins. Similarly, the ossa pterygoidea grow obliquely downwards and outwards by ventral 'external accretion', while their roots migrate 'unilaterally' so as to maintain the vertical position of each bone while broadening the soft palate. The processus pterygoidei move apart by laterally directed 'unilateral growth' which also enlarges the foramen orbitotundum. The foramen ovale and canalis alaris grow outwards and backwards by 'internal erosion' and enlarge very considerably as they do so.

In adult mice the corpus thickens enormously to contain sinuses, and the canalis pterygoideus is developed. They are absent in 3-week-old mice.



Text-fig. 13. Growth of the sphenoidale aborale; (a), cerebral aspect; (b), vertical section.



Text-fig. 14. Growth of the sphenoidale orale; cerebral aspect.

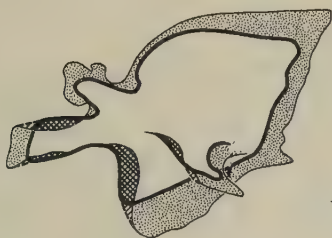
(iii) *Sphenoidale orale* (Pls. 7-9 and Text-fig. 14)

As mentioned when describing the changing orientation of the foramen magnum, the skull is not symmetrical in its growth processes. Similar asymmetry in the rates of growth of the bones in the base of the skull behind and in front of the sphenoidale orale cause this bone to leave the position it occupies at birth—the globular part of the cranium—and to move forward into the narrow part of the cranium. So that, besides growth processes which lead merely to the bones' enlargement, there are others which alter the bones' shape to suit the new environment.

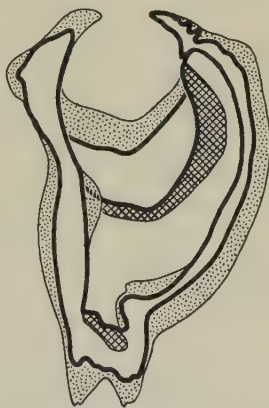
The corpus is lengthened by 'diaphyseal growth' at each end. Erosion of the sides of the back half of the corpus enlarges the foramen orbitotundum, while in

front it maintains the shape of the foramen opticum. 'Diaphyseal erosion' of the upper surface of the corpus, anteriorly, helps to flatten the bone out from the curved infantile condition.

At birth, the alae orbitales consist each of two rami which grow out in the 'diaphyseal' pattern from the corpus. Erosion of the sides bordering the foramen opticum results in the latter's enlargement. Normally at the third day of post-natal development the rami fuse laterally to enclose the foramen opticum. Thenceforward, alar growth is by 'external accretion' and 'internal erosion'. These processes continue right down the roots of the alae so that the roots continue to separate. In changing to their new position the anterior and posterior rami are brought to the same level by 'unilateral' growth in the vertical plane; while the inter-alar angle is reduced by graded rates of 'unilateral' growth which increase from their medial to their lateral margins. Owing to the first of these trends the foramen opticum comes to occupy a more horizontal plane which necessitates heavy erosion of all its margins so that the nervus opticus may continue to pass freely out of the skull.



Text-fig. 15. Growth of the squamosum; lateral aspect.



Text-fig. 16. Growth of the tympanicum; superior aspect.

A quite frequent feature of the mutants is that they have open optic foramina, in which respect they are comparable to 2-day-old normal mice. This condition may be due to inhibition of accretion owing to failure to enlarge the incisura sphenoidalis of the frontale, again suggesting the interdependence of bones for their normal growth.

#### (iv) *Squamosum* (Pls. 6-9 and Text-fig. 15)

The squamosum grows by 'external accretion' over almost all its margins, but particularly along the antero-inferior border. However, the processus postglenoidalis grows by 'diaphyseal growth' and the lower posterior border of the squamosum is heavily eroded for enlarging the fissura squamotympanica.\*

Erosion of the cerebral surface is confined peripherally and is associated with the extension of sutural overlaps with the parietale, frontale, ala temporalis and

\* Greene's (1935) 'postglenoid foramen'.

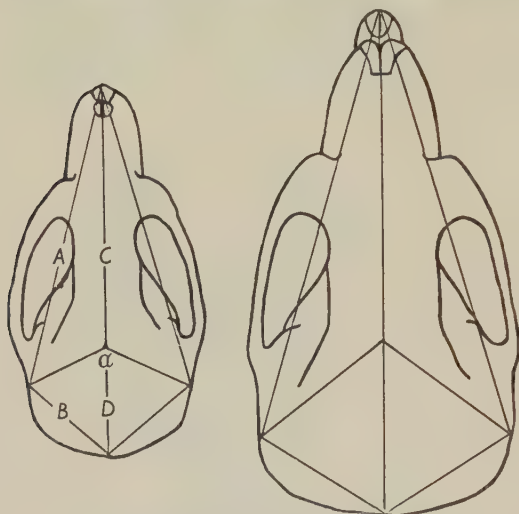


also with the 'diaphyseal growth' of both the processus postglenoidalis and processus caudalis.

Although passively maintaining its level on the squamosum the processus zygomaticus is carried up the side of the skull, partly by the relatively greater accretion along the inferior border of the squamosum, and also by 'external accretion' to the alae temporales of the sphenoidale aborale. The forward migration of the processus zygomaticus, however, is produced by its own 'unilateral growth'.

(v) *Tympanicum* (Pls. 7-9 and Text-fig. 16)

The bulla tympanica bulges ventrally, posteriorly and ventro-medially by 'external accretion' and 'internal erosion' which are particularly active in these directions. The tuba auditiva ossea (Eustachii) grows medially by 'external accretion'.



Text-fig. 17. Diagram showing how rotation of the bullae tympanicae (i.e. reduction of the angle  $\alpha$ ) is brought about by differential rates of growth fore and aft, both laterally and medially (i.e. of  $A/B$  compared with  $C/D$ ).



Text-fig. 18. Growth of the perioticum; inferior aspect.

On the seventh and tenth days after birth ossification spreads by 'internal accretion' from the posterior and anterior corners of the annulus tympanicus respectively into the porus acusticus externus. The porus of the retarded mutants is therefore exceptionally wide and has rough margins due to the infiltration of the blastemic tympanicum by bone spicules. When its ossification is completed the porus grows by 'internal erosion'.

The bulla is rotated in the course of its development, not from intrinsic processes, but by differential rates of growth between the mid-lines and the sides of the floor of the skull, in front of and behind the bulla (Text-fig. 17).

(vi) *Perioticum* (Pls. 7, 8 and 11 and Text-fig. 18)

'External accretion' occurs over almost all the visible surfaces of the perioticum. But as it is a hollow bone of great complexity, especially within the cochlea,

'internal erosion' must play a highly important part. Erosion is visible externally also; the base of the processus stylomastoideus moves posteriorly by 'unilateral growth', while its tip grows rapidly after delayed ossification by 'external accretion' within the perfectly modelled cartilage. The lateral margin of the foramen stylo-mastoideum is subject to 'internal erosion', and the posterior end of the sulcus stapedius is subject to superficial 'unilateral growth', the tegmen tympani grows downwards by 'unilateral growth', and the fossa parafloccularis is enlarged by 'internal erosion'. The fenestrae cochleae and vestibuli are first formed in bone by the flow of ossification around them followed by 'internal accretion', but this temporary phase is already over by 10 days, after which they are enlarged by the opposite process of 'internal erosion'. The very variable shape, but not necessarily the size, of the fenestra flocculi (N.B.) is determined by the extent of deficient initial ossification, which is only just completed in this region at 10 days.

(vii) *Malleus* (Pls. 8 and 10 and Text-fig. 19)

The mouse malleus is an extraordinarily large bone in which, relative to man, the size of the collum and processus longus (anterior) are enormously exaggerated. At 21 days the processus longus is firmly ankylosed with the tympanicum.

The growth of the malleus is characterized by a number of local torsions of one part of the bone relative to another. 'Unilateral growth' results in the twisting of the manubrium away from the processus longus in both ventral and lateral directions, and in the lateral twisting of the ventro-posterior part of the collum relative to its dorsal part. Medial accretion to the capitulum with medial erosion of the root of the processus longus (whereby it is converted into the collum) result in a fourth torsion. 'Diaphyseal growth' is involved in the anterior extension of the processus longus, in the antero-posterior broadening of the collum, and in the ventral growth of the processus muscularis.

(viii) *Incus* (Pl. 10 and Text-fig. 20)

The corpus grows by 'external accretion' and by the incorporation of the roots of the crura longum and breve. The crus longum grows ventrally by 'diaphyseal growth' with a mild, anteriorly directed 'unilateral' migration of its middle region. The lenticulare and its supporting processus lenticularis maintain their position at right angles to the tip of the crus longum by downwardly directed 'unilateral growth'. The crus breve grows obliquely downwards and posteriorly by 'external accretion', but the crest on its lateral side is bent into an S-shape by 'unilateral' movements in opposed directions at its two ends.

(ix) *Stapes* (Pl. 10 and Text-fig. 21)

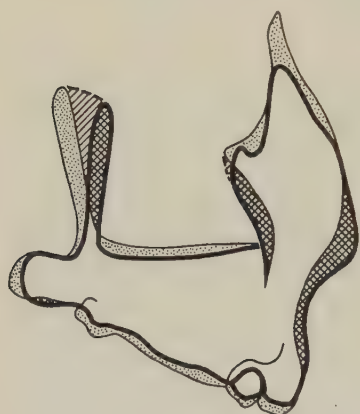
The stapes grows by 'external accretion' at more or less equal rates around its periphery; and 'internal erosion' of the foramen stapedis (N.B.) which maintains the thin crescent-like section of the crura and basis. The oddity of the shape of the mutant bone follows entirely from the gradual spread of ossification without erosion from the basis to the capitulum.

(x) *Interparietale* (Pls. 6 and 8 and Text-fig. 2b)

Growth of the interparietale in the transverse axis of the skull is 'centrifugal'. There is also 'external accretion' at its anterior margin and 'external erosion' at

its posterior margin. In the mutants, failure of erosion may cause it to override the squama occipitalis at its ventro-posterior corners.

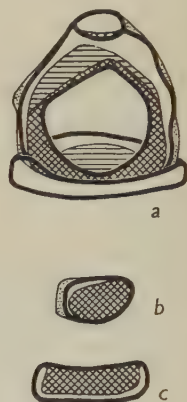
A remarkable feature of this bone's development is the inversion of the usual relationship between the rates of accretion and erosion at opposite margins, so that the interparietale may be larger in the long axis of the skull at 8 days than it is when adult. In this bone, therefore, 'external erosion' proceeds more rapidly than 'external accretion'. Table 1 shows the relative dimensions of the interparietale and the skull in two normal mice, one of them 3 weeks old, the other adult. The measurement in the younger mouse is taken as the unit for the corresponding measurement in the older. It is seen that the changes in the relative sizes of the skull and the interparietale are brought about more by the diminution of the interparietale than by the excess growth of the skull.



Text-fig. 19.



Text-fig. 20.



Text-fig. 21.

Text-fig. 19. Growth of the malleus; medial aspect.

Text-fig. 20. Growth of the incus; lateral aspect. Inset, growth of the processus lenticularis; posterior aspect.

Text-fig. 21. Growth of the stapes, (a) medial aspect, (b) cross-section of the crus anterium, (c) cross-section of the basis. (Horizontal shading indicates deep erosion of the surface seen in optical section.)

Table 1. *Relative lengths in older and younger normal mice of the skull and interparietale*

	Length of	
	Skull	Interparietale
Normal: 3 weeks	1	1
Normal: adult	1.21	0.69

(xi) *Parietale* (Pls. 6, 8 and 12 and Text-fig. 22)

The parietale grows entirely by the 'centrifugal pattern'. In so doing it gradually changes from the highly convex bone which extends well down the sides of the young animal's skull into the older animal's much flatter bone which is confined almost entirely to the roof of the skull. According to the balance at its periphery



between the opposing processes of accretion and erosion within this pattern so the bone changes its outline. At its periphery erosion predominates:

anteriorly on the medial side, so as to make way for the backward extension of the frontale,

on the whole of the lateral side to make way for the upward growth of the squamosum,

and posteriorly near the middle, in relation to remodelling of the sutura lambdoidea.

On the other hand, accretion is dominant:

posteriorly, at the medial corner, and

anteriorly, on the lateral side of the processus frontalis (N.B.).

There is no medial growth at the suture with the other parietal bones.

In the mutants, inhibition of this growth pattern sets up such stresses that mutant skulls burst with high frequency in the parietal region during their preparation.



Text-fig. 22. Growth of the parietale; internal or external aspect.



Text-fig. 23. Growth of the frontale; interna aspect.

(xii) *Frontale* (Pls. 6-8 and 12 and Text-fig. 23)

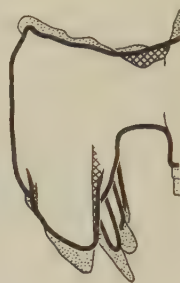
The pars nasofrontalis, or horizontal lamina of the frontale, is lengthened by 'centrifugal growth', i.e. by peripheral accretion to the external surface of its anterior and posterior margins, with peripheral erosion of the cerebral surface in these regions. Along the sutura coronalis the rate of backward accretion increases towards the mid-line, to some extent compensating for the erosion of the parietale in this region. As part of this 'centrifugal pattern' the arcus superciliaris is raised and sharpened by accretion to the outer, dorsal surface with compensating erosion of the cerebral surface. The processus zygomaticus grows forwards, sideways and upwards and bears the arcus zygomaticus with it away from the side of the cranium. This growth is associated with 'diaphyseal erosion' of the posterior border of the processus zygomaticus whence the arcus superciliaris is modelled. There is no

medial growth, but lateral accretion in the middle of the arcus superciliaris both widens the frontale and reduces the curvature of the orbit.

The pars orbitotemporalis (or vertical lamina) grows by 'external accretion' to all its borders with the exception of a little erosion of the anterior border of the incisura sphenoidalis; the latter otherwise being enlarged by the regression of ventral accretion away from its mouth. The crista ethmoidalis (N.B.) grows obliquely downwards and forwards, while 'diaphyseal erosion' scoops out the lower end of its anterior wall, and 'unilateral growth' whisks the upper 'tail' of the crest forwards. The foramina frontale (N.B.) and ethmoidale move forwards in the 'reversed unilateral pattern'; and migration of superficial localized sites of accretion and erosion, following accretion along the anterior margin, moulds the fossae frontales. Failure to enlarge the foramina frontale and ethmoidale may be partly responsible for the death of the mutants through constriction both of the veins supplying a transverse blood sinus of the brain, and of the nervus ethmoidalis.



Text-fig. 24. Growth of the interfrontale;  
lateral aspect.



Text-fig. 25. Growth of the  
ethmoidale; dorsal aspect.

(xiii) *Interfrontale* (Pls. 6 and 9 and Text-fig. 24)

The interfrontale is an ephemeral bone in the mouse though a constant feature of both the microphthalmic and grey-lethal stocks. According to Keeler (1933), on inadequate data, it is probably inherited as a single recessive. It consists of a stout caput (N.B.) articulating with the ethmoidale below, and a dorsoventrally flattened pars caudalis (N.B.).

In young mice the bone is flexed in accordance with the flexure of the skull roof in this region, and it becomes straightened out by upwardly directed 'unilateral growth' of the pars caudalis. The caput grows forwards in the 'diaphyseal pattern'.

(xiv) *Ethmoidale* (Pl. 12 and Text-fig. 25)

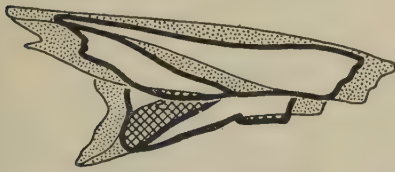
After fusion of the labyrinthi and lamina perpendicularis growth is in general by 'external accretion' and 'internal erosion', but with other small-scale processes maintaining the finer details of construction of the labyrinthi. Before fusion, the stalks of the labyrinthi grow towards the lamina perpendicularis by 'diaphyseal growth'. More detailed description of the ethmoidale's growth is impossible owing to the great variation in its form. (The cellulae ethmoidales are opened by fragmentation on disarticulating the skull.)

(xv) *Lacrimale* (Pls. 6, 8 and 11)

The capitulum (N.B.) grows upwards by 'diaphyseal accretion', and there is intensive 'diaphyseal erosion' of the anterior and ventral sides of the 'collum' (N.B.). The growth of the squama, however, cannot be described because of the inflection in the mutants of largely extrinsic deformities by its constriction within the unenlarged canalis infraorbitalis.

(xvi) *Nasale* (Pls. 6, 8 and 11 and Text-fig. 26)

The nasale grows in length by 'centripetal growth'; the 'unilateral' component in the middle of the bone leading to its progressive specialization as a roofing bone by converting it from a quartered cylinder (the shape determined by its terminal growth anteriorly) into a flatter and more horizontal sheet. This component also carries the sutural articulation for the incisivum (premaxilla) forwards so that it maintains its position relative to the growing tip.



Text-fig. 26. Growth of the nasale and concha dorsalis; lateral aspects.



Text-fig. 27. Growth of the vomer; lateral aspect.

(xvii) *Concha dorsalis* (Pl. 11 and Text-fig. 26)

By analogy with the nasale with which it ankyloses at 9 days, the concha dorsalis grows forwards by 'external accretion'. In addition, there is 'diaphyseal growth' posteriorly. The intensive 'diaphyseal erosion' of the lateral side transfigures the solid posterior block into the dainty longitudinal anterior lamella. The foramen perforating this lamella is carried backwards by 'reversed unilateral growth'.

(xviii) *Vomer* (Pl. 11 and Text-fig. 27)

The mouse vomer consists of a basis (N.B.) which rests on the processus palatini of the maxillae, and two large alae vomeris. These comprise (i) the alae septales (N.B.) which are situated immediately above the basis and lie vertically on either side of the cartilaginous septum nasi; and (ii) the horizontal alae ethmoidales (N.B.) which are the posterior extensions of the alae vomeris behind the basis. They cup the labyrinthi of the ethmoidale and fragment on their separation. Hence their ragged appearance.

The basis grows forwards and downwards by 'unilateral growth'. The alae septales grow forwards by 'external accretion', the alae ethmoidales backwards and downwards, also by 'external accretion'. Because the labyrinthi do not separate to any great extent, the angle between the elongating alae ethmoidales becomes progressively smaller. This is achieved by erosion of the medial sides of the roots of the alae, accompanied by lateral accretion. The backs of the alae septales are converted into the roots of the alae ethmoidales by 'unilateral' growth processes in the horizontal plane.



(xix) *Incisivum* (Premaxilla) (Pls. 6-8 and Text-fig. 28)

Posteriorly, the incisivum gains length and height by 'external accretion' to the processus nasalis and palatinus, and to the corpus. While anteriorly there is no increase in length, there is a further contribution to the height of the bone, such that the bone more than compensates for the removal of the nasale from the side of the skull (*vide supra*) and adds considerably to the height of the skull in this region. There is no medial growth.

The only complications visible externally are the backward migration by 'diaphyseal growth' of the crest demarcating the anterior and medial limit of the canalis infraorbitalis; the enlargement of the mouth of the alveolus incisivus superior by 'internal erosion' of its anterior, lateral and posterior, but not medial margins; and the backward extension of the corpus at the expense of the fissura palatina, by 'internal accretion'.

Of the processes affecting the interior of the bone, only those affecting the eruption of the incisor tooth are discussed.



Text-fig. 28. Growth of the incisivum; inferior aspect.



Text-fig. 29. Growth of the maxilla; left, inferior aspect; right, superior aspect.

*Eruption of the dens incisivus.* While in the mutant mice the incisor tooth is trapped in the corpus of the incisivum, in normal mice extremely extensive 'internal erosion' of the alveolus allows the root of the tooth to grow backwards, right out of the incisivum and into a special cup developed by the maxilla. This backward growth of the tooth is associated with a considerable thickening of the lateral wall of the incisivum by 'external accretion' to both its medial and lateral surfaces. Moreover, anteriorly 'internal erosion' enlarges the diameter of the alveolus by removing part of its lateral, posterior and anterior walls, thus enabling the tooth to erupt through the gums.

(xx) *Maxilla* (Pls. 6-8 and 13 and Text-fig. 29)

The main trends of growth in the maxilla are upwards and laterally; there is little medial growth and negligible accretion to the under side.

While the tip of the processus zygomaticus grows backwards, sideways and slightly upwards simply by 'external accretion', the more anterior regions of the processus shift sideways and also slightly upwards by 'unilateral growth'. It is

noteworthy that some parts of the processus must be eroded and rebuilt many times before the bone doubles in thickness. The vertical flange below the most anterior parts of the processus zygomaticus is cut out of it by superficial 'external erosion' of the crista facialis. The flange forms the side wall of the canalis infraorbitalis, which is enlarged by the 'unilateral growth' of the flange beside it; and of the processus frontalis above it. There is no erosion of its ventral or medial walls.

The lamina orbitalis grows backwards and upwards and the lamina infraorbitalis grows forwards and upwards, both by 'external accretion' to their periphery. Between them, the incisura lacrimalis is formed by 'diaphyseal erosion' of their adjoining borders. Their basal portions are shifted sideways by 'unilateral growth' which also causes the expansion of the fossae maxillares (N.B.).

The delicate cup which houses the tip of the root of the upper incisor grows by accretion to all but its lateral surfaces while being deeply hollowed from in front by 'internal erosion'. Failure of the latter may account for the flatness of this portion of the alveolus in the mutants (cf. inhibitory action in the manubrium mallei (p. 220)).

There is some medial accretion to the processus palatinus, while 'diaphyseal erosion' enlarges the fissura platina in both medial and posterior directions. The lateral border of the fissura, however, is not eroded, while enlargement of its anterior regions is cared for by the incisivum. Growth of the processus in length is by 'centripetal growth', the 'unilateral' component of which lowers the roof of the palate. The notch for the foramen palatinum majus is widened by 'internal erosion' of the medial border of the processus palatinus and of the base of the lamina orbitalis. 'Internal accretion' takes part in its backward migration.

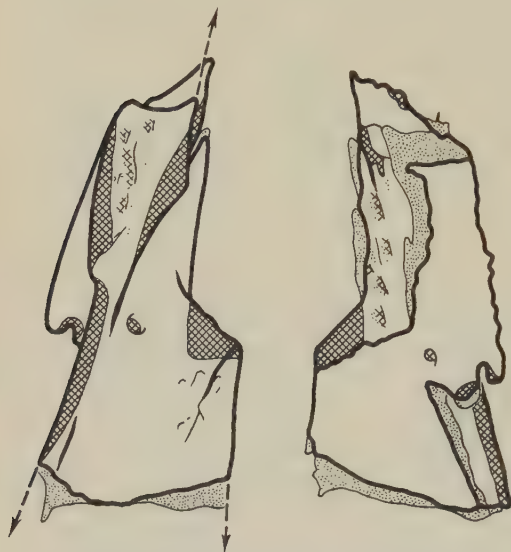
On the limbus alveolaris, which grows at equal rates forwards and backwards, the 'rough' for articulation for the 'palatinum' is probably maintained by backwardly migrating alternating zones of superficial erosion and accretion. In the mutants, failure of erosion in the palatinum probably accounts for the mal-development of the crests by accretion to the opposite surface of the maxilla, and vice versa.

*Eruption of the molar teeth.* The molar teeth are at first largely passive in their eruption, which results from processes involving the maxillary bone about them. First, the foramina above the roots of the teeth are closed by 'internal accretion' thus completing the process of ossification of the maxilla. Then the incurved walls of the alveoli are eroded so that the teeth can be carried out of the crypt by accretion within and by the continued growth of their own roots. The roof of the crypt is lowered by 'external erosion', and with the passage of the bulky crowns from the body of the maxilla the limbus alveolaris becomes much shallower and narrowed down. These processes continue into old age so that in old mice the teeth are rooted in a very broad and shallow bed which encroaches on the palate to a quite considerable extent.

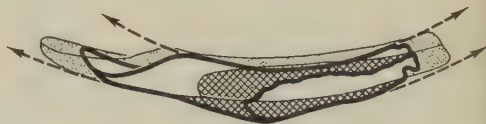
(xxi) *Palatinum* (Pls. 7 and 13 and Text-fig. 30)

The palatinum grows in length equally at each end. This growth is 'diaphyseal'. Anteriorly, 'diaphyseal erosion' cuts out the anterior border of the foramen palatinum majus and remodels both sides of the processus orbitalis; posteriorly it enlarges the foramen sphenopalatina by cutting down the processus sphenopalatinus, and shortens the lamina pteryopalatina. The meatus nasalis is widened by 'dia-

physeal accretion' to the medial border of the pars horizontalis and by 'unilateral erosion' of the base of the pars perpendicularis; it is heightened by 'diaphyseal erosion' of the nasal surface of the pars horizontalis and by 'unilateral erosion' of the pars orbitalis. The pars horizontalis is also widened by accretion to its lateral border. The 'roughs' for articulation with the maxilla, sphenoidale orale and alae temporales are maintained by the migration of alternating zones of very superficial accretion and erosion. The foramen palatinum minus migrates obliquely backwards and laterally by 'reversed unilateral growth'.



Text-fig. 30. Growth of the palatinum; left, superior aspect; right, inferior aspect.



Text-fig. 31. Growth of the zygomaticum; lateral aspect.

(xxii) *Zygomaticum* (Pls. 6-8 and Text-fig. 31)

Growth of the zygomaticum is 'centripetal'. Accretion at its ends progresses at about equal rates; and the erosional component not only reduces the curvature of its inferior border, but also extends the surface for articulation with the maxilla at the expense of the anterior limit of the corpus. As this trend is coupled with posterior accretion to the corpus this appears to slide backwards in relation to the processus maxillaris. There is some medial erosion in this region of the processus temporalis. Accretion to the lateral surface is the chief cause for growth in thickness.

In the mutants, the anterior end of the processus maxillaris is strongly flexed medially, but there is no sign either in mutants or in normals of medial erosion in this region. It is therefore concluded that this is an extrinsic phenomenon imposed on the mutant zygomaticum by the anomalous growth of the processus zygomaticus of the maxilla with which it is here in intimate contact.

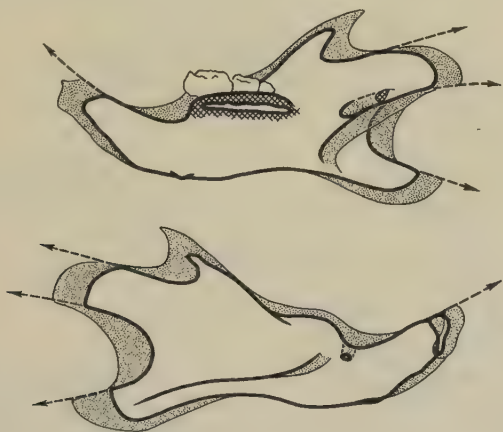
(e) THE LOWER JAW (Pl. 14 and Text-fig. 32)

In spite of the complexity of its shape, erosion enters surprisingly little into the growth of the mandibula. As seen from the side, its shape is almost entirely



maintained by differential rates of peripheral accretion. Generally the rate of growth anteriorly is less than that posteriorly; and while almost the whole of the upper border is a site of upward growth, growth downwards is limited to the posterior end. Apart from the merest suspicion of erosion over a small section of the margo interalveolaris, the remaining borders are either accretional or 'indifferent'.

In the backward growth of the processus condyloideus there is 'diaphyseal erosion' of the lateral side of the collum above its mid-line, and on the whole of its medial surface. The foramen mandibulare is carried backwards by accretion to its 'trailing' edge though a little erosion of its leading edge is also required. By accretion to its ventro-posterior border the processus condyloideus incorporates the root of the processus angularis.



Text-fig. 32. Growth of the mandibula; medial aspect above and lateral aspect below.

Erosion is not manifest elsewhere except in the enlargement of the alveoli of the teeth. However, for the molar alveoli it is only transient, for after the release of the teeth by removal of the incurved walls of the alveoli, the alveolar margins become sites of active accretion, while there is even more accretion to the floor of the alveoli. Thus the crowns of the teeth are pushed above the level of the jaw, a remarkable process which has been observed both by Kölliker (1873) in the ox and by Brash (1934) in the pig. At the same time the molar teeth with their alveoli slide slowly forwards and laterally by the latter's 'unilateral growth'. Perhaps no better account of eruption of the mandibular molars can be found than in Hoffman & Schour's study (1940*b*) in the rat. From their study it can be seen that the growth of the tooth root by accretion of both dentine and secondary cementum is also important in bringing about occlusal movement of the tooth throughout life. The potential increment to the 'clinical crown' of the tooth is, however, exactly balanced by attrition if occlusion is normal. In the alveolus incisivus inferior 'internal erosion' is very important; for by its activity the incisor tooth can extend from its confines in the corpus (its location at birth) right under the dentes molares, almost to the foramen mandibulare. In so doing, the tooth becomes very much wider and there is consequently much 'internal erosion' even at the mouth of the alveolus. So great is the pressure of tooth growth in the mutants that their incisor roots often force

their way into the canalis mandibularis and thence out of the foramen mentale. Here they form a calcified misshapen lump (Grüneberg, 1935) round which the jaw grows, so leaving a 'crater' which is a frequent characteristic of both microphthalmic and grey-lethal mice.

The reader interested in the growth of the teeth themselves is referred to Hoffman & Schour (1940*b*) and Grüneberg (1937).

The lateral side of the jaw is the site for greatest contribution to growth in breadth, although, as the incisor root tunnels through the pars molaris there is medial accretion here too. The crista masseterica is moved forwards slightly by accretion to its more anterior slopes, while the foramen mentale changes the direction of its opening from sideways to upwards by lateral accretion directly below it.



Text-fig. 33. Growth of the hyoideum; inferior or superior aspect.



Text-fig. 34. Growth of the scapula.

#### (f) HYOID (Pl. 11 and Text-fig. 33)

(i) The basis of the hyoideum grows in a slight dorso-ventral curve and with a much stronger antero-posterior curve by 'centripetal growth'.

(ii) Proximally, the cornu majus grows 'diaphyseally', while posteriorly it is at 3 weeks still infiltrating its cartilaginous model by 'external accretion'.

(iii) At about 10 days, ossification of the cartilaginous cornu minus begins; at 3 weeks it is only just completed. The manner of its later growth is not known though it is probably by 'external accretion' too.

#### (g) FORE-LIMBS AND GIRDLE

##### (i) Scapula (Pl. 15 and Text-fig. 34)

The most rapid growth in the scapula occurs along its margo vertebralis. So triangular is the blade that there is little 'diaphyseal erosion' of either the margo cervicalis or margo axillaris; but on its lateral surface 'diaphyseal erosion' on either side of the spina scapulae hollows out the vertebral limits of the fossae supra- and

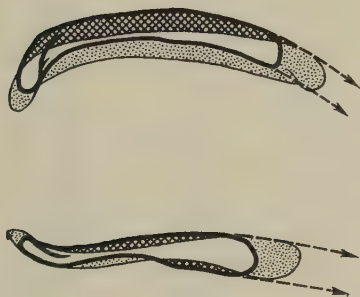
*infra-spinata*. On the facies medialis 'diaphyseal erosion' plays a very unimportant role. There is a little accretion near the *cavitas glenoidalis*.

The *spina scapulae* grows 'unilaterally', the erosional component enlarging the *incisura spinoglenoidalis* while the un-eroded parts become the root of the *acromion*.

The *acromion* ossifies later than the *collum* and grows by 'diaphyseal accretion' to its ventral border at a rate intermittent between those for the *margo vertebralis* and *cavitas glenoidalis*. Only its cervical margin is eroded.

The scapula increases in height by accretion to the free edge of the *spina scapulae* and *margo axillaris*, and by unilateral growth of the *acromion*.

The *coracoideum*, which fuses with the scapula at 18 days, grows only by external accretion.



Text-fig. 35. Growth of the clavicula; above, posterior view; below, lateral view.



Text-fig. 36. Growth of the humerus; posterior aspect.

## (ii) *Clavicula* (Pl. 15 and Text-fig. 35)

In the mouse, the *extremitas acromialis* (N.B.) which represents the 'acromial end' and 'conoid tubercle' of man, meets the corpus at the *angulus claviculae* (N.B.).

The clavicle grows in the 'centripetal' pattern with 'diaphyseal growth' of the *extremitas sternalis*.

## (iii) *Humerus* (Pl. 17 and Text-fig. 36)

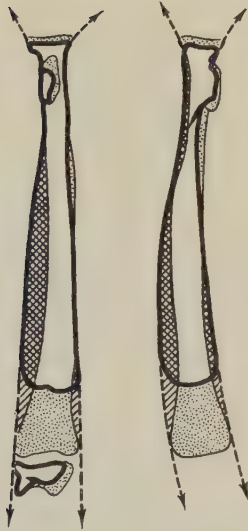
Practically all increase in length of the humerus results from 'diaphyseal growth' at the proximal end of the corpus associated with 'unilateral growth' of the *crista humeri* and *tuberositas deltoidea*. Towards its lower end the corpus is thickened by accretion to its antero-lateral surface, just as accretion to the free (antero-lateral) edge of the *crista* adds to its height.

The distal end contributes almost nothing to the bone's length, but it is generally enlarged. The *crista epicondylis lateralis* and its proximal continuation, the *margo*



lateralis, become increasingly prominent by accretion to their antero-lateral edges; and the former is thrown into greater prominence by slight 'diaphyseal erosion' of the anterior surface of the root of the epicondylus lateralis. The epicondylus medialis grows medially with 'diaphyseal erosion' of its distal border.

The caput and trochlea grow by 'external accretion' alone. The latter fuses with the diaphysis (corpus) at about 7 days.



Text-fig. 37. Growth of the radius; left, ventral view; right, medial view.



Text-fig. 38. Growth of the ulna; left, medial view; right, dorsal view.

(iv) *Radius* (Pl. 16 and Text-fig. 37)

Unlike the humerus, the radius grows much faster (eight times as fast) at its distal end than at its proximal end. Growth is 'diaphyseal', but the erosion is almost entirely limited to the dorso-medial surface and produces a curvature of the bone which does not result directly from its terminal accretion. This curvature is enhanced by 'unilateral' growth near the proximal extremity. The tuberositas radii (which contains the 'punctum fixum') enlarges by 'external accretion' to all its surfaces. The small amount of growth at the proximal end of the corpus is 'diaphyseal'. Neither epiphysis suffers erosion; the proximal epiphysis fuses to the collum at about 14 days.

(v) *Ulna* (Pl. 16 and Text-fig. 38)

In the ulna, proximal growth is a little more important, though distal 'diaphyseal growth' is still the chief contributor to growth in length. Erosion is more sym-

metrical than in the radius, though the distal end is eroded chiefly from its ventro-lateral side (again in contrast to the radius where the most intense erosion was from the dorso-medial side). Lateral erosion is important in shaping the tuberositas ulnae. Near the incisura semilunaris the sharp juvenile dorso-ventral flexion of the bone is reduced by 'external accretion' which is greater on the ventral side than on the dorsal surface.

The lips of the incisura grow upwards and away from one another with 'diaphyseal erosion' reforming the corpus behind their recurved margins.

Growth of the processus olecrani is by 'external accretion', while the olecranon (its epiphysis) is unilaterally eroded from its diaphyseal side.\*



Text-fig. 39. Growth of a typical metacarpal or metatarsal bone.



Text-fig. 40. Growth of a typical proximal phalanx; from above.



Text-fig. 41. Growth of a typical terminal phalanx; from the side.

#### (vi) *Manus* (Pl. 17)

The drawings of the manus were made from alizarin clearances of the intact hand; and as it was impossible to orientate all of the bones correctly with regard to the other members of the comparative series, the following analyses have been essentially rule-of-thumb. However, as such (provisional) analyses have previously been borne out by the conclusions from the more precise method of superimposing outline drawings, considerable confidence may be placed in the present series.

*Carpus*. Ossification of the cartilaginous carpals does not begin until the third day after birth and then proceeds by 'external accretion' alone. No mutant carpal shows any anomaly which might be attributed to erosional failure. It is not known for what period this simple method of growth is maintained.

*Metacarpus* (Text-fig. 39). With the exception of the metacarpale primum whose growth is more typical of a phalanx, the metacarpales grow by 'diaphyseal growth' of the distal end of the corpus; with 'external accretion' to the capitulum and basis, neither of which contribute greatly to the bone's growth in length.

*Phalanges proximales* (Text-fig. 40). (Phalanges prima and secunda of digiti 2-5; and phalanges prima only of digitus 1.) 'Diaphyseal growth' of the *proximal* end

\* This is the only occasion in which 'unilateral growth' of epiphyses (a general phenomenon in the pig (Payton, 1933)) was observed in the present work.

of the corpus; with 'external accretion' to the capitulum and the basis, which fuses with the corpus at about 15 days after birth. There is very little 'diaphyseal erosion' of the plantar surface of the corpus.

*Phalanges terminales* (Text-fig. 41). (Phalanx tertia of digiti 2-5; and phalanges secunda of digitus 1.) Ossification spreads proximally from the tuberositas unguicularis and growth continues by 'external accretion' to the articular surface with the capitulum phalangis proximalis. The foramen transversarium phalangis (N.B.) migrates backwards by 'reversed unilateral growth'; and the distal root of the bony spicule which bridges it is also eroded.

*Ossa sesamoidea*. The growth of these bones is by 'external accretion' alone.

#### (h) HIND-LIMBS AND GIRDLE

##### (i) *The pelvis* (Pl. 18 and Text-fig. 42)

The right and left halves of the mouse pelvis, the ossa coxae, are readily separated. In adult male mice a few spicules extend across the symphysis pelvis (symphysis ossis pubis) (Ruth, 1936) and in 3-week-old mice the symphysis is still cartilaginous. In pregnant females it is represented by a ligament which may be as much as 2-3 mm. wide. In virgin and in non-pregnant females the ligament is much narrower, but there is never bony communication.

Each os coxa itself consists of four bones. Three are large, the ossa ilium, pubis and ischii, but the fourth, the os acetabuli, is extremely small. These four bones are more or less separate structures until they fuse at about 3 weeks in the acetabular region; the pubis and ischii fuse posteriorly about 10 days after birth. The four bones are treated individually in this account.

The rate of growth of the three major bones is much less at their acetabular extremities than at their ends distal to the acetabulum. In the course of their development, the acetabular sutures are remodelled from a tiradiate figure, Y, into a  $\gamma$ -like figure, associated with a slight overlapping of the bones.

*Ilium*. (While retaining the veterinary term tuba coxae (Ellenberger & Baum, 1926) for the ventral edge of the ala ossis ilium, the author has shifted the positions of the spina iliaca anterior superior and the spina iliaca anterior inferior caudally so as to conform with their muscle attachments in man, and has translated these terms into forms more suitable for four-footed animals. The translations are as follows:

the spina iliaca anterior superior becomes spina iliaca ventralis cranialis, and the spina iliaca anterior inferior becomes spina iliaca ventralis caudalis; and to bring their analogues on the dorsal side of the ala into line, the spina iliaca posterior superior becomes spina iliaca dorsalis cranialis, and the spina iliaca posterior inferior becomes spina iliaca dorsalis caudalis.)



Text-fig. 42. Growth of the pelvis (os coxa); lateral view.



While the growth at both ends of the ilium is 'diaphyseal', the erosional component is limited to the outer surface (facies glutea) anteriorly, and to the inner surface (facies pelvina) posteriorly. Anteriorly, the 'diaphyseal' erosion helps to cut out the tuber sacrale which migrates forwards in the 'unilateral pattern'. Along the ventral margin of the bone, however, accretion is directed both downwards and forwards, so that the 'unilateral pattern' is not involved in the forward migration of the spina iliaca ventralis caudalis.

The rate of growth at the acetabular extremity is never great and falls off until its cessation with the ossification of the acetabular sutures at 3 weeks.

*Pubis.* Like the ilium, the pubis grows at very unequal rates at its two ends, the slower growth at the acetabular end ceasing altogether at 3 weeks. Growth at each end is 'diaphyseal', but the erosion is restricted to three sites, one to each end of the obturator border, the third to the facies pelvina near the junction of the rami acetabularis and symphysicus. The bone is deepened by 'external accretion' to its ventral border, so obliquely directed that neither the eminentia iliopectinea nor the tuberculum pubicum involves 'unilateral growth' in their divergence movement in the antero-posterior axis of the bone.

Owing to the opposition of accretional and erosional sites in the pubis the ramus symphysicus can be regarded as growing 'unilaterally' backwards; and the ramus acetabularis as growing 'unilaterally' downwards.

*Ischii.* Like the other pelvic bones, the ischium grows very little at its acetabular end. Distally, growth is 'diaphyseal' with heavy erosion of the acetabular border and some mild erosion of the facies pelvina of the ramus symphysicus. At the acetabular end there is accretion to both the ilial and pubic sutures (contributing to growth in length and breadth respectively) with 'diaphyseal erosion' of the buttress for the facies lunata and of the pelvic surface. Between the two sites of 'diaphyseal growth' the corpus is thickened by accretion to both dorsal and obturator borders.

*Acetabuli.* The os acetabuli, which forms the pubic wall of the acetabulum, grows only by 'external accretion'.

(ii) *Femoris* (Pls. 18 and 19 and Text-fig. 43)

Four-fifths (80%) of the growth in length is contributed by the 'diaphyseal growth' of the distal extremity of the corpus. Erosion is very intense on the ventral surface where it removes the ridges on either side of the fossa intercondyloidea, producing the flat planum popliteum.

With the exception of the obliquely medial and upward 'diaphyseal growth' of the collum femoris, growth at the proximal end of the bone proceeds by 'external accretion'. The distal migration of the trochanter tertius is obtained without erosion, by discrepant rates of accretion to its distal and proximal edges.

(iii) *Ossa cruris (tibia and fibula)* (Pl. 19 and Text-fig. 44)

In the tibia and fibula terminal growth is slightly faster proximally than distally. This growth is 'diaphyseal', but in the fibula it interacts with strong 'unilateral' trends. The crista tibiae is enlarged by accretion to its anterior and lateral edge, so that in very old animals it becomes curved back upon itself. On either side of the crista tibiae 'diaphyseal erosion' spreads with increasing intensity to the

posterior side of the tibia, where it serves to enlarge the spatium interosseum cruris. This is also enlarged by the bowing out of the fibula posteriorly by a 'unilateral' trend which almost obliterates 'diaphyseal erosion' of its posterior surface.

At the distal ends of the bones 'diaphyseal erosion' is limited to the medial side of the tibia and to the lateral side of the fibula. And the fibula gradually becomes incorporated into the tibia by the building up of the posterior side of the tibia to the level of the fibula and by erosion of the posterior surface of the fibula.



Text-fig. 43. Growth of the os femoris; dorsal view.



Text-fig. 44. Growth of the tibia and fibula; (a) anterior, (b) medial, and (c) posterior view.

The juvenile flexions of the bones are softened by changes in their middle regions. In the tibia there is accretion to posterior, lateral and medial surfaces with some erosion anteriorly. In the fibula there are 'unilateral' movements directed medially towards the extremities and laterally in the intervening region.

The only pattern involved in the growth of the epiphyses is 'external accretion'.

(iv) *Patella* (Pl. 19)

The bony patella grows by 'external accretion' to the centre of ossification in the sesamoid cartilage which precedes it.

(v) *The pes* (Pl. 20)

(Unlike the domesticated animals and man, the mouse has three in place of two proximal tarsal bones. Rather than regard the supernumerary bone as an unparalleled entity, the author agrees with Greene (1935) in believing this to be the

os tarsi tibiale, the 'talus' to be the os tarsi intermedium, and the 'calcaneum' to be the os tarsi fibulare).

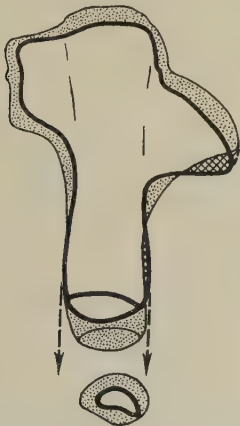
*The tarsus.* The growth of only the os tarsi fibulare (calcaneum) (Text-fig. 45) is more complicated than by 'external accretion' alone.

With the exception of the sustentaculum tali in whose forward growth the 'unilateral pattern' is involved, growth of the anterior end of the fibulare is by 'external accretion'. 'External accretion' also widens the girth of the middle of the bone; while, posteriorly, 'diaphyseal growth' contributes a further increase in length. Erosion of the lateral side is obliterated by accretion which introduces a slight curvature of the body of the fibulare. The tuber calcanei grows by 'external accretion'.

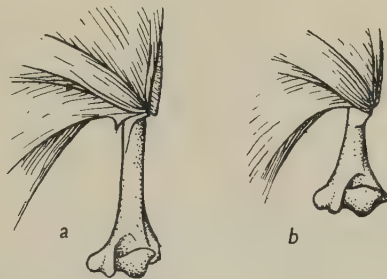
*Metatarsus and digiti pedis.* The growth patterns of the metatarsus, phalanges and ossa sesmoidea are precisely similar to those of the manus and require no further comment.

#### (i) PENIS BONE

The os priapis grows at its proximal end by 'diaphyseal growth'.



Text-fig. 45. Growth of the fibulare; ventral view.



Text-fig. 46. Dissections of the forearm of a normal mouse (a), and a microphthalmic mouse (b) at 21 days, showing the migration of the deltoid muscles along the crista humeri. Is the consequent release of muscular tension the normal stimulus for erosion of the crista?

#### (j) COMPARISON WITH OTHER AUTHORS' RESULTS

Providing there is a reasonable similarity in their form, there is, in general, a striking resemblance in the manner of growth of the bones of the mouse, described above, and of other animals (rat, cattle, pigs, man) described by other authors. In fact, the resemblance in the growth of the lower jaws of the pig, ox and mouse transcends their resemblance in shape.

However, there are some more serious discrepancies. In particular, while 'unilateral growth' has been found for only one epiphysis in the mouse (olecranon), Payton (1933) found it in all the twelve pigs' epiphyses which he studied; and in one epiphysis the effect of no less than four-fifths of the accretional increment was



simultaneously removed by erosion of the opposite surface. These differences may be related to age changes in the manner of growth of the bones.

Kölliker (1873) described additional sites of erosion on the medial side of the processus jugularis of the occipitale; on the upper surface of the processus zygomaticus of the squama temporalis, and on the medial side of the lacrimale. He also found anterior growth in the incisivum where the present author found only backward growth. In describing the growth of the humerus, Kölliker may have erred in failing to recognize the possibility of the migration of the deltoid muscles. Dr Grüneberg (unpublished) first noted the migration of these muscles up the crista humeri in sections of grey-lethal mice, and the present author has confirmed this in dissections of microphthalmic mice (Text-fig. 46).

Lastly, it may be remarked that the relative rates of growth at the ends of the six major long bones of the limbs vary from species to species, and in every case the rates described here for the mouse differed from those of the pig (Payton, 1932). The equality of growth at the two ends of the tibia and fibula, and the lack of growth at the distal extremity of the humerus, in mice, are, however, quite compatible with the manner of growth of these bones in humans as obtained by the analysis of Harris's radiographs (1933).

## DISCUSSION

### SOME GENERAL PROBLEMS IN BONE GROWTH

Having dealt with the details of the growth of individual bones, it is now time to discuss some more general problems of bone growth, and, in particular, those problems upon which the present work may throw additional light. It is intended chiefly to discuss the relative importance of inherent and extrinsic determination of bones and of bone growth, while also touching on the significance of the osteoclast and of the epiphysis.

#### (a) *Embryonic determination of bones*

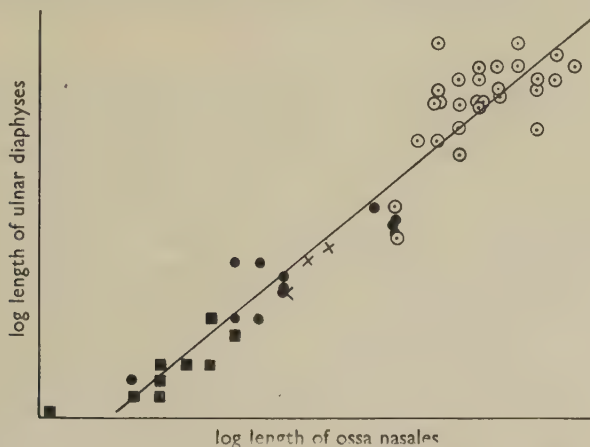
The modern concept of bone growth is vastly different from the theories of the early mechanists who believed bone to be laid down along lines of stress (the trajectorial theory) and erosion to be a necrotic process; and it is now known that bones have an identity quite apart from their normal environments. Thus Saunders (1948) proved that the bones of the chick's limbs were finally determined immediately following their serial proliferation from the apical ectodermal cap of the limb-buds. And in earlier experiments (notably those of Murray & Huxley (1925), Fell (1928) and especially Fell & Robison (1929)—all reviewed by Murray (1936)) the enormous power of explanted blastemic bones to self-differentiate in tissue cultures, even when stripped of their musculature, was demonstrated. Lastly, in a series of papers (1942-7) Lacroix has published accounts of the osteogenic properties of mere fragments of bone, and even of bone membrane (periosteum) and conjugation cartilage which could produce bone of normal histological appearance growing in a manner typical of the bone from which the pieces had been taken. Pfeiffer (1948) has demonstrated a similar property of marrow cells. On the other hand, Hauschka (1951) has gone too far in claiming to have found differentiated bones from mouse embryo mince injected intraperitoneally into adult mouse hosts, for while the shapes

of the 'bones' were comparable to normal bones, their manner of growth was not, and it would be better to interpret the objects as nothing more than osteomata. This type of research culminated in Lacroix's discovery (1947) that even an alcoholic extract of conjugation cartilage had osteogenic properties and was capable of inducing an osteoma growing in a more or less typical 'diaphyseal' manner.

These facts leave little scope for the play of normal environmental forces occurring within the developing animal in the determination of normal bone growth.

(b) *Accretion and extrinsic factors*

In spite of the degree of self-determination it is generally believed that the sites of accretion and erosion are not so independent of extrinsic factors (Murray & Selby, 1930) and come under the influence of specific stimuli. It is rarely possible to put the supposed stimuli for accretion to independent test, and in the one case



Text-fig. 47. Graph showing the constancy of the allometry of the length of the snout to an 'unrelated' body measurement in normal and mutant mice of 20 and 21 days and in three normal mice of only 14 days of age.  $\odot$   $+/+$ ,  $+/gl$  and  $+/mi$  at 21 days of age;  $\times$   $+/+$  at 14 days;  $\blacksquare$   $gl/gl$  at 20 and 21 days;  $\bullet$   $mi/mi$  at 21 days.

where this was possible, a non-specific factor was found responsible. Grüneberg had once suggested (1935, and quoted by de Beer, 1940) that the snout of the mouse required a stimulus from the growth of the incisor tooth to attain its proper length, and that the shortness of the grey-lethal snout was due to the mal-development of the incisor tooth. This idea is now definitely invalidated, for a plot of the length of the nasal bones (in lieu of the snout) against an 'unrelated' body measurement (corpus ossis ulnae) for grey-lethal, microphthalmic and 2- and 3-week-old normal mice (Text-fig. 47) shows that the regression line relating these measurements to each other is common to all the four groups. Thus the shortness of the grey-lethal snout is due merely to the general retardation of development and not to any circumstance peculiar to the region of the snout.

It is not at all clear to what extent other stimuli might be responsible for individual sites of accretion. But in one case at least it appears that the extrinsic factors have a more passive role: to provide *conditions* for general development, not an array *stimuli* for specific features of development. Thus in a remarkable

teratoma taken from a girl and containing the three middle digits of a 'hand', perfection in development was graded disto-proximally (Nicholson, 1937). The most proximal structure bore no resemblance to any known bone. This was followed by two lumps of bone and the three 'metacarpals' thinly ossified and fused at their proximal ends. The distal ends of the 'metacarpals' and the phalanges were almost normal and the terminal phalanges were lenticulated. It seems as if the existence of ever more proximal structures assisted towards the provision of more normal conditions for the distal structures subsequently delineated from the apical cap.

Now the importance of intrinsic factors in bone accretion does not mean that the skeleton is an anarchy of individually governed bones. On the contrary, such almost perfect correlations as between the measurements of the two bones above (Text-fig. 47) are not usually met in studies of qualitative characters (such as weight and tail length) and betray the existence of a factor which rigidly controls the rate of growth of all bones in the skeleton and is not intrinsic to any single bone.

(c) *Extrinsic factors affecting erosion*

While the extrinsic factors affecting accretion evade discovery, those affecting erosion are more apparent. Jores (1920) caused erosion of the processus spinosi of thoracic vertebrae in guinea-pigs and rabbits by exerting a pressure on them by tying little mercury-filled bags to them, or by strapping water bags under the skin. And Loeschke & Weinholdt (1922) have recorded instances in man of erosion of the inner tables of the skull leading to exposure of the diploë and even to fenestration, due to abnormally high internal pressures caused by hydrocephaly, brain tumours and premature synostosis of the skull. They also showed that erosion ceased and may be replaced by accretion when the pressure is removed. It is, however, natural that the bones surrounding such a delicate organ as the brain should be responsive to the organ's variations in form; and Loeschke's and Weinholdt's findings cannot necessarily be extended to other parts of the skeleton.

Indeed, it may well be that the release of tension is sometimes the stimulus for erosion. Thus, in sectioned material of grey-lethals Grüneberg (unpublished) found that the deltoideus and pectoralis muscles had migrated away from the tip of the crista humeri, whereas in normal mice their insertion coincides with the tip of the crista. (The present author has confirmed this anomalous situation in dissection of microphthalmic mice, and one of such dissections, with a normal for comparison, is figured in Text-fig. 46.)

Enthusiasm for investing pressure with the responsibility of causing erosion must be tempered by consideration of Payton's work (1933) concerning the rates of accretion and erosion at opposite surfaces of the articular epiphysis of the long bones of the limbs of the pig. In all of the twelve epiphyses he studied he observed the 'unilateral' pattern of growth, and although the pressure at articular and diaphyseal surfaces of each epiphysis must have been approximately equal, erosion of the diaphyseal surface in one epiphysis was four-fifths as great as accretion to the articular surface. Nor was there an obvious correlation between the rates of erosion of adjacent epiphyses. In other words, pressure cannot be the *cause* of erosion. At most it can only be a stimulus to which a given bone or part of a bone may or may not respond; and emphasis is again laid on the intrinsic properties of the individual bones.



It was mentioned in the Introduction that the skeletons of grey-lethal and microphthalmic mice were not quite identical and that the microphthalmics frequently showed less obvious signs of erosional failure. And it was once thought (Grüneberg, 1948*b*) that a comparative study of the skeletons of these mice might furnish further information on the problem of the control of erosion. Alas, it is now clear that a semblance of erosion in these mutants can be produced merely by differences in the time of onset and in the rate of ossification. For, in the mutants, remodelling progresses normally until the 'bone' becomes calcified. Thus, if ossification of the stapes is delayed, the size of its foramen stapedis is more normal; and if ossification, once initiated, is rapid, the mutant stapes will present fewer of its characteristics which result from the slow progress of ossification from basis to capitulum. On the other hand, the slow progress of ossification in the tibia will produce more gracefully shaped metaphyses which might be interpreted as indicating a partial success at erosion. It would, therefore, seem impossible to draw any reliable conclusion concerning effective stimuli for erosion from the intended study.

As for the intrinsic control of erosion, this seems to lie with the conjugation cartilage for, whereas dead bone is totally absorbed on transplantation (Hancox, 1947), the presence of conjugation cartilage (or its contaminants), as in Lacroix's experiments, leads to regulated erosion. But how this influence of the conjugation cartilage is transported throughout the bone is not known. Hancox (1949) has suggested that the occurrence of erosion is determined by trophic gradients; but here it should be noted that, whereas in 'diaphyseal growth' much of the youngest bone is absorbed, in 'unilateral growth' it is the oldest.

*(d) Plasticity in patterns of growth*

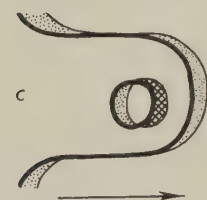
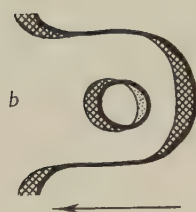
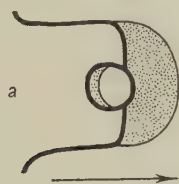
The topic of the changeability, or plasticity, of the manner of growth of bones during their development is especially applicable to the problem of extrinsic versus intrinsic determination of sites of both accretion and erosion. There are few bones indeed (some carpal, tarsal and sesamoid bones) which adhere throughout their life to a single pattern of growth ('external accretion');\* and even in these, shortly after ossification has begun, internal changes occur in the bones which are not visible from the exterior and are, therefore, not considered in this paper. But in every other bone even the externally visible patterns of growth change from time to time.

Thus in the arcus of the vertebrae, 'external accretion' (during the ossification of the original cartilaginous model) is soon replaced by 'centripetal growth' which is itself replaced by a tertiary pattern ('external accretion' with 'internal erosion') when the elements of the vertebra fuse into a single unit. Similar radical alterations in patterns of growth must always occur whenever bones are involved in bony fusions—as when the occipital bones, or the sacral vertebrae, or the pelvic bones, or the tympanicum and perioticum and malleus, or epiphyses and their diaphyses unite.

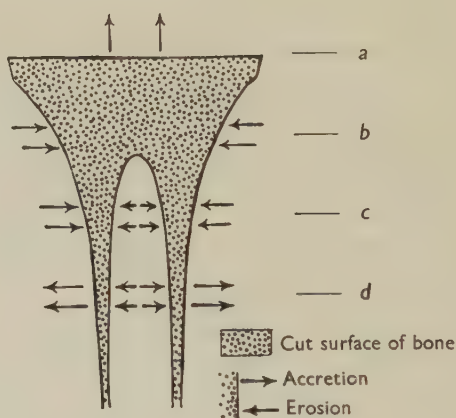
\* Schour & Massler (1940) and Massler & Schour (1951) have also noted that there are two phases of growth: the first of generalized accretion, followed by a second and longer period of only localized accretion. The first period of growth lasted up to 60 days in the rat. This conclusion, however, may be erroneous, since it appears that these authors did not realize that while alizarin (which they were using to record the bones' manner of growth) stains only the growing bone in old skeletons, it stains bone formed long previous as well as just after its injection in young animals (Cameron, 1930).

In the tympanicum the tertiary pattern is a reversal of the secondary. 'External accretion' from the annulus tympanicus is followed by 'internal accretion' in the porus acusticus externus; but when the ossification is completed (by 14 days after birth) the porus is enlarged like the rest of the bulla, by 'internal erosion'.

The foramen transversarium likewise undergoes a reversal of secondary and tertiary patterns (Text-fig. 48*a-c*). Its ossification is accomplished by 'internal accretion' (*a*), which is replaced by medially directed 'reversed unilateral growth' as part of the 'centripetal growth' of the arcus vertebrae (*b*); but when the vertebral elements fuse and their growth is by 'external accretion' with 'internal erosion', the direction of 'reversed unilateral growth' of the foramen is also reversed (*c*).



Text-fig. 48. Plasticity of bone growth. I. The three successive patterns of the process transversus.



Text-fig. 49. Plasticity of bone growth. II. The four successive patterns of growth affecting a piece of a long bone initially at (*a*).

It is instructive to consider the fate of a single disc of bone adjacent to the conjugation cartilage of a long bone (Text-fig. 49). As it is carried towards the middle of the bone by continued calcification of the conjugation cartilage, it may be subjected to no less than four successive patterns of growth. At first (*a*) it is subjected to 'diaphyseal accretion' by ossification of the matrix of the conjugation cartilage (*endochondral* ossification). Then when the disc comes to occupy a position a little behind the cartilage its circumference is 'diaphyseally' eroded (*b*). This 'diaphyseal erosion' is accompanied by a continuous *endosteal* reformation of the wall of the metaphysis between the *endochondral* spicules (Leblond *et al.* 1950).

A little later (c) it is also internally eroded for the extension of the marrow cavity. And, lastly (d) when it is very near the middle of the bone it partakes in the widening of the diameter of the diaphysis by 'external accretion' (*periosteal ossification*), while 'internal erosion' continues to enlarge the marrow cavity. Thus, in a long bone these four processes and these three types of bone formation may be depicted as migrating away from the punctum fixum towards the growing ends, each successive phase tending to encroach on each previous phase—a dynamic picture truly reflecting the plasticity of bone growth.

It seems impossible to attribute such radical alterations in the patterns of growth of adjacent regions at the same time, and of the same region at adjacent times, to equally fundamental changes in the local environmental factors which are sometimes presumed to control the bone's growth. And it seems preferable to turn our attention away from supposed (extrinsic) stimuli for growth and erosion towards the study of the bone's (intrinsic) reaction to its environment. But extrinsic factors are not entirely banished from influencing bone growth: to them are bequeathed those abnormalities of development which allow, for example, a skilled anatomist to recognize the skeleton of a cobbler; a bone to form ball-and-socket joints on either side of an unsplinted fracture; and the cessation or arrest of growth with severe illnesses.

But if difficulty is encountered in attributing the plasticity of bone growth to an ever-changing array of environmental factors determining local processes, then a similar difficulty must be encountered if this array is substituted merely by an array of *inherent* factors. In short, it is probable that the inherent determination is not of local growth processes but of whole patterns of growth. Thus the conjugation cartilage does not only produce endochondral bone, but also controls the extent of metaphyseal erosion, the extension of the marrow cavity, the formation of marrow, and the deposition of periosteal bone (Lacroix). Similarly, fracture of the shaft of a bone is not associated merely with the local deposition of new bone at the site of repair, but stimulates the deposition of bone over almost the entire surface of the shaft (Brooks, 1917).

#### (e) *The function of the osteoclast*

Now bone accretion and bone erosion are not just opposite processes in the same reversible reaction, but are quite distinct processes. Bone accretion is a function of cartilage and periosteal cells and a third type of cell called osteoblasts; but erosion is the function of a fourth type of cell called the osteoclast—though, as the relation between osteoclast and erosion has been open to doubt, it will be discussed here. In the light of the present contribution it is no longer permissible to accept the indecisive conclusion which Hancox (1949) reached after a comprehensive review of published research on the osteoclast—that in relation to bone absorption 'osteoclasts must still be regarded as enigmatical structures'.

In the first place it is no longer possible to regard the osteoclast as unrelated to the process of bone absorption, since the sites of erosion described by the present author closely tally with the sites of osteoclasts described by Kölliker (1873).

Next, the osteoclast cannot be a by-product of erosion (an opinion held as late as 1937 by Wilton), since it occurs with almost normal frequency in the grey-lethal



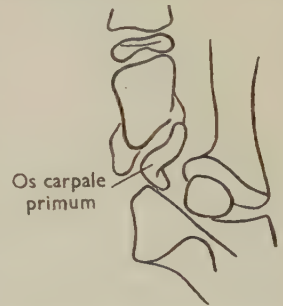
mouse (Barnicot, 1947) in which there is no bone erosion at all. Moreover, Kölliker has reported seeing osteoclasts even before ossification centres had been formed.

But are we to believe with Jaffe (1933) that the osteoclast merely removes bone cells, or that it actually erodes the bone salts? Of this there can be no final proof without microcinematographic recording of the action of the osteoclast (Hancox, 1949). However, the wide fluctuations in the population of osteoclasts mentioned in the Introduction (p. 216) allow no store to be placed in Ruth's objection (1937) that the number of osteoclasts which he observed at the symphyseal tables of the ossa pubes of the pregnant guinea-pig was too small to account for the extent of erosion there. On the other hand, the close correspondence in size between the foveolae of Howship and the osteoclasts which they contain is strong evidence of the erosive action of osteoclasts on bone. Since no evidence of bone phagocytosis has been obtained (McClean & Bloom, 1941) it is almost certain that this dissolution is by the application of extracellular osteolytic enzymes.

(f) *The phylogenetic determination of bones*

In spite of the lack of ontogenetic plasticity in bone determination there is still widespread adherence to Broom's (1930) belief in the phylogenetic transmutation of bones. Broom believes the os carpal primum to represent the metacarpale primum of therapsid reptiles, and the metacarpale primum of living mammals to be a transformed phalanx. On the other hand, owing to the close resemblance (see Text-fig. 50) of the 10-day-old os carpal primum to a typical proximal phalangeal epiphysis (basis phalangis), the transformation could be argued differently. But any theory which attempts to explain the distribution of the epiphyses in the hands and feet in such a manner presupposes two postulates: first, that the ossified elements in the hands and feet are permanently represented throughout phylogeny; and secondly, that the distribution of the epiphyses is determined by extrinsic factors varying along the proximo-distal axis of the limb. Neither of these postulates can be considered proven.

No living tetrapod can supply evidence concerning the first postulate, as it is characteristic of this whole group for the first digit, when present, to have at least one element less than the others. But the evidence of the fossil, mammal-like reptiles, stresses the ephemerality of the elements of the hands and feet, for whole segments of the digits and carpus have disappeared in the course of evolution without being retained as transformed bones; and conversely, they have appeared without being converted from an already existing bone. Thus the Pelycosaurians have three more carpal bones than the mouse and four supernumerary phalanges; the Gorgonopsids are similar but lack the os carpal 5 and some intermediate phalanges are greatly shortened; while the Dicynodonts, though having the typical mammalian phalangeal formula, lack the os carpal 5 but possess the mammalian os carpal accessorium. Moreover, the ephemerality of epiphyses is clearly demon-



Text-fig. 50. The os carpal primum of a 10-day-old normal mouse. Note its resemblance to a typical phalangeal epiphysis

strated by reports in human pathology. For Burke (1930), Ogilvie (1931*a*), Pryor (1936) and Brailsford (1948) have all observed cases of first or second metacarpal bones with proximal and distal epiphyses in place of the normal single epiphysis. Brailsford has also noted phalanges with two epiphyses, and Ogilvie (1931*b*) records a case of a bilaterally symmetrical bipartite os carpale radiale. None of these authors comments on or figures correlated deficiencies in the rest of the skeleton of the hand so that it is clear that epiphyses can be created *de novo*.

Pryor's observations (1936) on the bifurcated and completely split thumbs of two children suggest that the epiphyses of the metacarpal bones are determined, not by a proximo-distally graded field (the necessary second postulate for accord with the theory of evolutionary transmutability of the elements of the hands), but by a transversely graded field. For in the case of bifurcation, the postaxial process (that nearest the metacarpale secundum) had a distal epiphysis as well as the common proximal one; while the preaxial process was without the distal epiphysis. Similarly, in the case of complete separation, the postaxial segment had no proximal epiphysis, while the preaxial segment had. (The radiographs were not clear enough to determine the disposition of distal epiphyses.)

However, Carter (1951) notes instances in the mouse of a splitting and even complete bifurcation or trifurcation of the big toe associated with the gene *luxate*, in which it is the *preaxial* digits which exhibit triphalangy and have a distal epiphysis. Carter regards the extra digits as prehallucal. In view of this evidence, if the distribution of epiphyses (and the number of phalanges) is determined simply by a transverse gradient, then it would appear that the gradient originates in the true hallux itself.

It may, nevertheless, be concluded that the similarity of the first metacarpal and metatarsal bones to phalanges, both in regard to the position of their epiphyses and to the manner of their growth, is not an indication of their common phylogeny.

#### (g) *The significance of epiphyses*

The epiphysis is an anatomical entity about whose evolutionary significance there has been much confused thought owing to the misconception that it is peculiar to mammals. Haines (1938) has shown that epiphyses are present as cartilaginous structures in bony fishes and Amphibia, and that the evolution of ossified epiphyses has occurred independently in reptiles and mammals (Haines, 1941). Theories which are concerned with the appearance of epiphyses in mammals only, are therefore discredited.

We have just seen in the special case of the mammalian hand and foot that the epiphyses are unlikely to represent pre-existing bones in phylogeny ('atavistic' epiphyses of Parsons, 1903), and we must therefore turn to their possibilities as functional entities. Epiphyses occur only at the growing ends of cartilage bones and transmit either articular pressure or muscular traction to the shaft (Parsons, 1904, 1905). In situations where there is neither mobile articulation nor muscular traction, as at the costo-chondral junctions or symphysis spheno-occipitalis, no epiphyses are formed. Haines (1940) has discounted Parson's theory that traction epiphyses are derived from sesamoid structures, and Appleton (1922) has shown that, unlike sesamoid bones, these epiphyses develop independently of their

musculature. It appears, then, that all epiphyses are formed by the separation of the growth cartilage into epiphyseal and conjugation cartilage, the latter becoming subterminal and retaining its contribution to growth (Harris, 1933). What advantages does this confer?

While traction epiphyses quite obviously present an economy in the amount of migration of muscle attachments which would be necessary were the growth cartilage terminal, the significance of pressure epiphyses is not as clear. From his study of the epiphyses of primitive forms Haines (1938) believed that the division of labour of the growth cartilage allowed the elaboration of the articular surface without interfering with the development of an efficient trabecular pattern in the shaft which demanded a flat growth cartilage. However, in higher animals the conjugation cartilage has become so infolded (Text-fig. 51) that it is questionable whether this advantage has been maintained. For the higher animals a more plausible suggestion is due to Nicholson (1937), that the situation of the growth cartilage behind the elaborately modelled and enlarged head presents a great saving in the extent of bone remodelling. This theory cannot apply, however, to the simple epiphyses of primitive forms; and it therefore appears that the advantages gained by the development of epiphyses have changed in the course of their evolution.



Text-fig. 51. Oblique view of the distal end of the shaft of the femur to show the infolding of the conjugation cartilage.

#### (h) *Conclusions*

It can be seen from the foregoing discussion that there still remain many unsolved problems in bone growth. These have been sufficiently stressed, but it is proposed to bring this subject to a close by drawing together some of the more significant positive conclusions which have been attained.

Bones are considered to be determined almost entirely independently of extrinsic control; yet the co-ordination in their rates of growth implies the existence of a common extrinsic growth-controlling factor. They appear to possess an extraordinary lack of variability both in ontogenetic and in phylogenetic determination; the distribution of the epiphyses of the metacarpale and metatarsale primum is not considered evidence of their evolution from phalanges, nor is the carpale primum regarded as a modified metacarpal bone or phalangeal epiphysis. The value of the epiphysis seems to have changed in the course of its evolution.

Patterns of growth of individual bones in the mouse may change no less than four times (often with severe contrasts between successive patterns) in the course of the first 3 weeks of the bone's formation. This, too, reflects the almost total lack of extrinsic control of bone growth. The conjugation cartilage is the centre of control of 'diaphyseal growth' of long bones, and the osteoclast is demonstrated the agent of erosion.

Murray & Selby's conclusion that extrinsic factors become of increasing



importance in advanced development of the bone, is modified; to extrinsic factors is now relegated the control only of abnormal growth.

#### SUMMARY

1. The uses of gene actions to replace operative technique in experimental embryology are discussed; and the two mutant stocks of mice used for the present study are described.

2. Methods of studying bone growth are discussed, and a new method is described and is used in the present study. This is due to Grüneberg (1948), and involves the interpretation of the condition of hereditary pathological skeletons.

3. The superficial versus interstitial nature of bone growth is considered.

4. The growth of the mouse skeleton in terms of patterns of accretion and erosion at normally visible surfaces is described. Eight patterns are described as fundamental in bone growth. The general results of earlier workers in this field are confirmed.

5. The relative importance of extrinsic and intrinsic factors in bone determination and bone growth are discussed. Extrinsic factors are considered responsible only for abnormal growth.

6. The distribution of epiphyses in the metacarpus and metatarsus is shown to be due to a transversely graded field and cannot indicate the evolution of the metacarpale primum or metatarsale primum from a proximal phalanx.

7. In spite of the rigidity of developmental and evolutionary determination of bones, their manner of growth is immensely plastic and many change four times within the early development of the bone.

8. While the significance of the epiphysis is still uncertain, the osteoclast is proved to be the agent of erosion.

The anatomy of the normal skeleton of the mouse at the age of three weeks is depicted in a series of half-tone, camera lucida drawings. Standard latinized nomenclature is used throughout.

The author is deeply indebted to Dr Hans Grüneberg for suggesting this problem and for the interest he has shown throughout its investigation. He is especially grateful to Mr E. D. Roberts for tuition in drawing, and for the elaborate care he has employed in labelling these drawings, and for assistance with the text-figures. He wishes to thank Dr D. S. Falconer for much helpful advice on writing the text; and Prof. C. H. Waddington for granting him permission to continue this work in Edinburgh. Part of the cost of publication of the plates has been met by a grant from the Carnegie Trust which is gratefully acknowledged.

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#### EXPLANATION OF PLATES

The skeletal anatomy of normal and grey-lethal mice at 3 weeks of age: features of normal bones are labelled. Original drawings made by camera lucida at standard magnification of  $\times 9.5$ , with the exception of vertebrae coccygeae, palatinum, lacrimale and patella (magnification of  $\times 19$ ), and auditory ossicles ( $\times 30$ ). All drawings reduced in reproduction to two-thirds, i.e. to  $\times 6.5$ , 13 or 20.

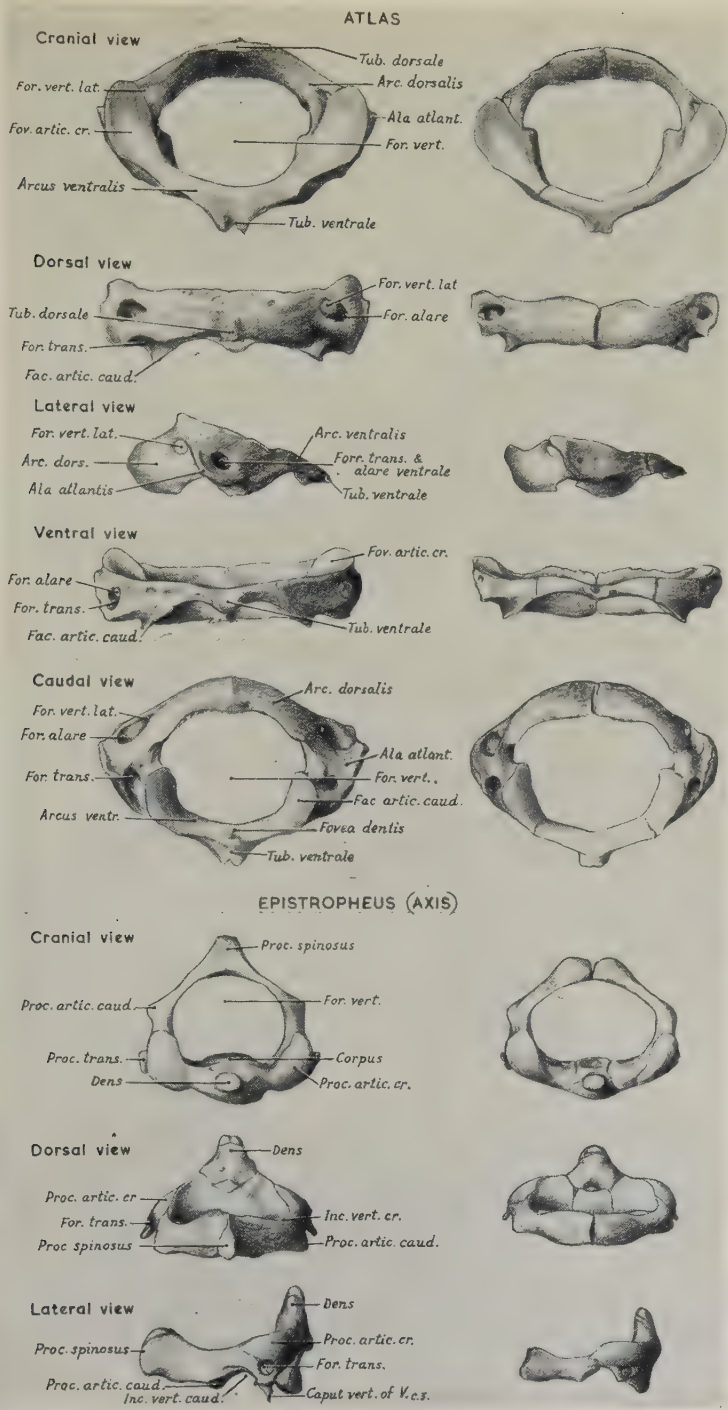
#### Index of abbreviations used in the plates

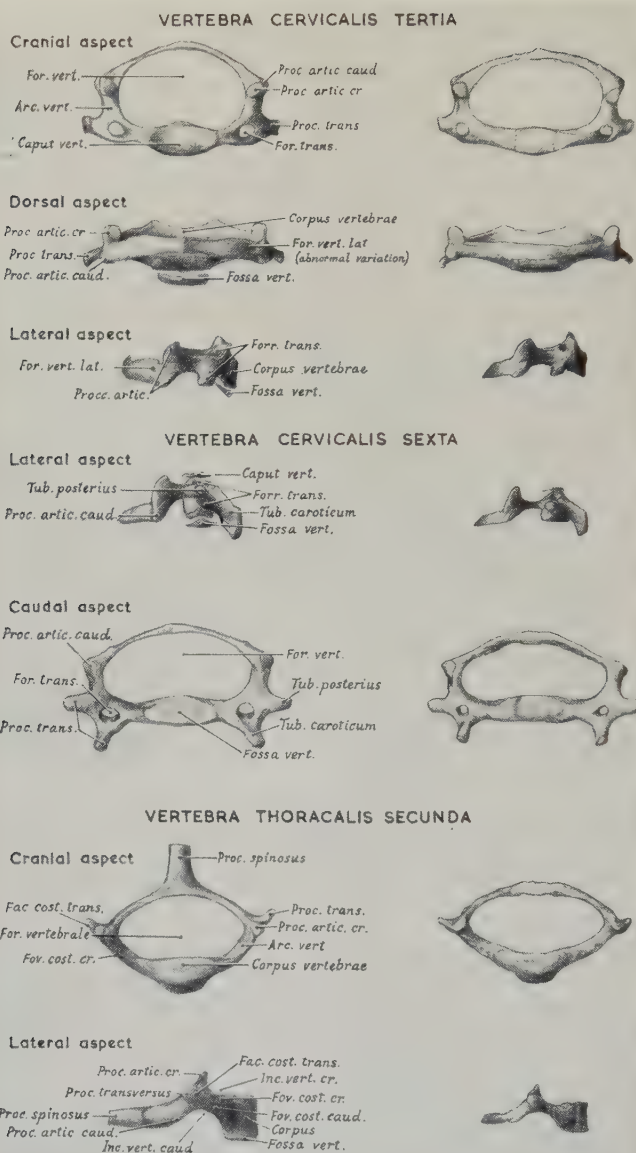
<i>Ala atlant.</i>	Ala atlantis
<i>sac.</i>	sacralis
<i>temp.</i>	temporalis
<i>Alv. inc. sup.</i>	Alveolus incivus superior
<i>Arc.</i>	Arcus
<i>dors.</i>	dorsalis
<i>vert.</i>	vertebrae
<i>Arcus ventr.</i>	ventralis
<i>Can.</i>	Canalis
<i>infr.</i>	infraorbitalis
<i>pteryg. (ant. opening)</i>	pterygoideus (anterior opening)
<i>Capit.</i>	Capitulum
<i>cost.</i>	costae
<i>mand.</i>	mandibulae
<i>Caput vert. (of V.<sub>c.3</sub>)</i>	Caput vertebrae (of vertebra cervicalis tertia)
<i>Cartil. scap.</i>	Cartilago scapulae
<i>Coch. tali prox.</i>	Cochlea tali proximalis
<i>Coll.</i>	Collum
<i>Cond. med.</i>	Condylus medialis
<i>lat.</i>	lateralis
<i>Corpus vert.</i>	Corpus vertebrae

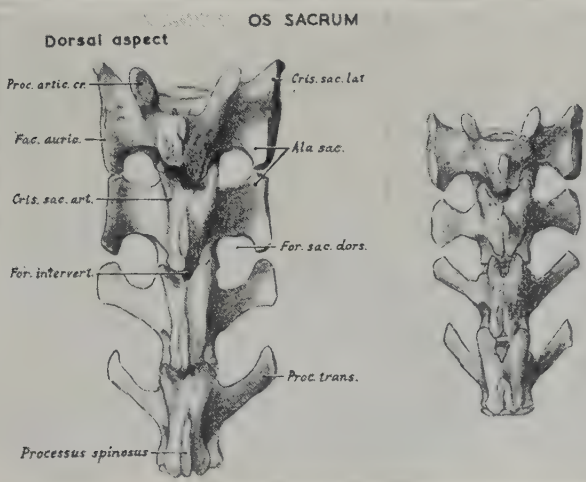
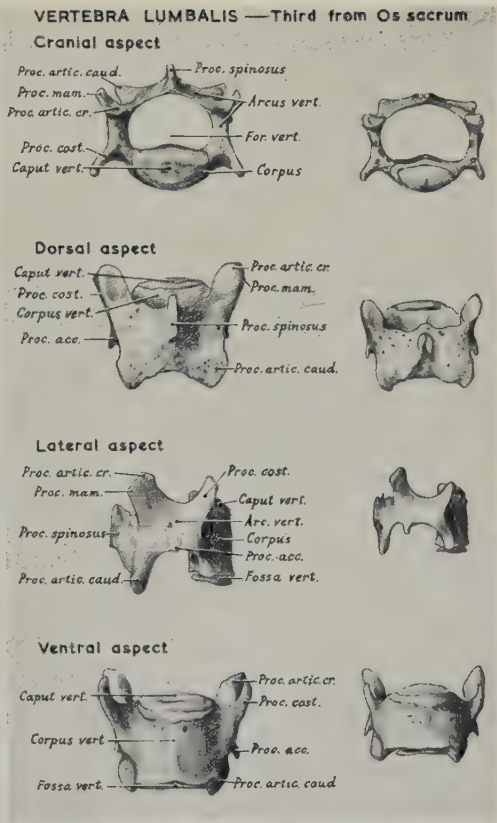
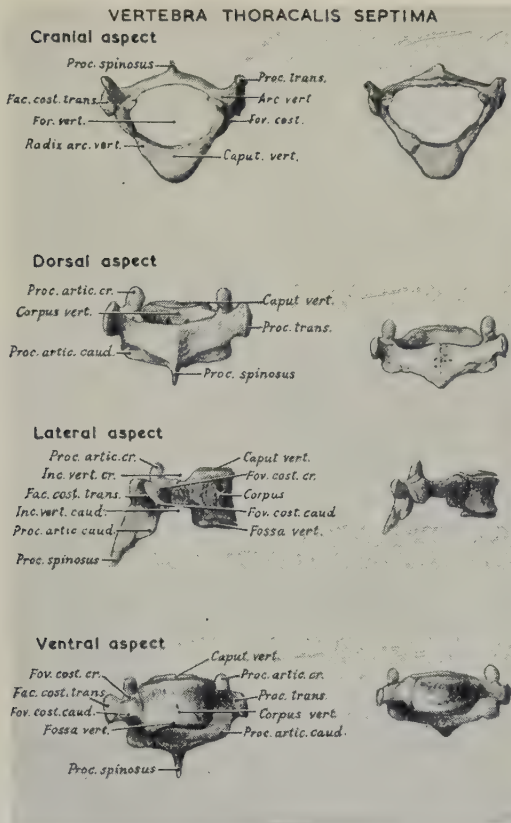
<i>Cris.</i>	<i>Crista</i>
<i>epic. lat.</i>	epicondyli lateralis
<i>eth.</i>	ethmoidalis
<i>sac. art.</i>	sacralis articularis
<i>sac. lat.</i>	sacralis lateralis
<i>tub. min.</i>	tuberculi minoris
<i>D. m. tertius</i>	<i>Dens molaris tertius</i>
<i>Emin. iliopect.</i>	<i>Eminentia iliopectinea</i>
<i>intercond.</i>	intercondyloidea
<i>Epic. lat.</i>	<i>Epicondylus lateralis</i>
<i>med.</i>	medialis
<i>Extr. acr.</i>	<i>Extremitas acromialis</i>
<i>dist.</i>	distalis
<i>st.</i>	sternalis
<i>vert.</i>	vertebralis
<i>Fac. artic. caud.</i>	<i>Facies articularis caudalis</i>
<i>auric.</i>	auricularis
<i>cost. trans.</i>	costalis transversarium
<i>Fen.</i>	<i>Fenestra</i>
<i>Fiss. pal.</i>	<i>Fissura palatina</i>
<i>For.</i>	<i>Foramen</i>
<i>intervert.</i>	intervertebrale
<i>lac.</i>	lacerum
<i>nutr.</i>	nutricium
<i>obt.</i>	obturatum
<i>pal. maj.</i>	palatinum majus
<i>sac. dors.</i>	sacrale dorsale
<i>sac. vent.</i>	sacrale ventrale
<i>stylomast.</i>	stylomastoideum
<i>trans.</i>	transversarium
<i>trans. phal.</i>	transversarium phalangis
<i>vert.</i>	vertebrale
<i>vert. lat.</i>	vertebrale laterale
<i>Forr.</i>	<i>Foramina</i>
<i>fr. int.</i>	frontalia interna
<i>pal. min.</i>	palatina minora
<i>trans.</i>	transversaria
<i>Foss. max.</i>	<i>Fossae maxillares</i>
<i>Fossa infrasp.</i>	<i>Fossa infraspinata</i>
<i>mand.</i>	mandibulae
<i>mass.</i>	masseterica
<i>supratr.</i>	supratrochlearis
<i>vert.</i>	vertebrae
<i>Fov. artic. cr.</i>	<i>Fovea articularis cranialis</i>
<i>cost.</i>	costalis
<i>cost. caud.</i>	costalis caudalis
<i>cost. cr.</i>	costalis cranialis
<i>Inc.</i>	<i>Incisura</i>
<i>acet.</i>	acetabuli
<i>clav.</i>	clavicularis
<i>lacr.</i>	lacrimalis
<i>sem.</i>	semilunaris
<i>sph.</i>	sphenoidalis
<i>spinoglen.</i>	spinoglenoidalis
<i>vert. caud.</i>	vertebralis caudalis
<i>vert. cr.</i>	vertebralis cranialis
<i>Incc.</i>	<i>Incisurae</i>

<i>Lam.</i>	Lamina
<i>infr.</i>	infraorbitalis
<i>pt-pal.</i>	pterygopalatina
<i>Lin. anc.</i>	Linea anconaea
<i>intertr. post.</i>	intertrochanterica posterior
<i>Linea trans.</i>	Linea transversa
<i>M.</i>	Margo
<i>Os c.</i>	Os carpi
<i>c. acc. pr.</i>	carpi accessorium primum
<i>c. acc. sec.</i>	carpi accessorium secundum
<i>pteryg.</i>	pterygoideum
<i>ses. ph. tertiae</i>	sesamoideum phalangis tertiae
<i>sph.</i>	sphenoidale
<i>tarsi inter.</i>	tarsi intermedium
<i>Ossa sess. ph. pr.</i>	Ossa sesamoidea phalangis primae
<i>Pars horiz.</i>	Pars horizontalis
<i>nasofr.</i>	nasofrontalis
<i>orb.</i>	orbitotemporalis
<i>perp.</i>	perpendicularis
<i>Pect. o. pubis</i>	Pecten ossis pubis
<i>Ph.</i>	Phalanx
<i>Proc.</i>	Processus
<i>acc.</i>	accessorius
<i>artic. caud.</i>	articularis caudalis
<i>artic. cr.</i>	articularis cranialis
<i>condyl.</i>	condyloideus
<i>corac.</i>	coracoideus
<i>cost.</i>	costarius
<i>mam.</i>	mamillaris
<i>palat.</i>	palatinus
<i>pteryg.</i>	pterygoideus
<i>sphenopal.</i>	sphenopalatinus
<i>stylomast.</i>	stylomastoideus
<i>trans.</i>	transversus
<i>zyg.</i>	zygomaticus
<i>Procc. artic.</i>	Processus articulares
<i>R. acet.</i>	Ramus acetabularis
<i>sym.</i>	symphysicus
<i>Radix arc. vert.</i>	Radix arcus vertebrae
<i>Sp. il. dors. caud.</i>	Spina iliaca dorsalis caudalis
<i>il. dors. cr.</i>	iliaca dorsalis cranialis
<i>il. ventr. caud.</i>	iliaca ventralis caudalis
<i>il. ventr. cr.</i>	iliaca ventralis cranialis
<i>nas. ab.</i>	nasalis aboralis
<i>Spatium inteross. cruris</i>	Spatium interosseum cruris
<i>Sulcus stap.</i>	Sulcus stapedeus
<i>Sut.</i>	Sutura
<i>Sym.</i>	Symphysis
<i>p.</i>	pubis
<i>Syn</i>	Synchondrosis
<i>intersph.</i>	intersphenoidalis
<i>T. isch.</i>	Tuber ischiadicum
<i>Tab. isch.</i>	Tabula ischiadica
<i>Tub.</i>	Tuberculum
<i>cost.</i>	costae
<i>Tubs.</i>	Tuberositas
<i>delt.</i>	deltoidea
<i>infragl.</i>	infraglenoidalis
<i>supragl.</i>	supraglenoidalis





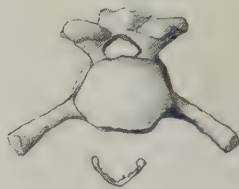
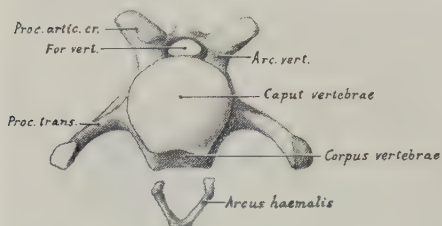




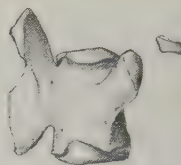
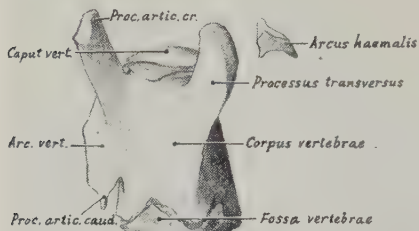


VERTEBRAE COCCYGEAE  
The fourth

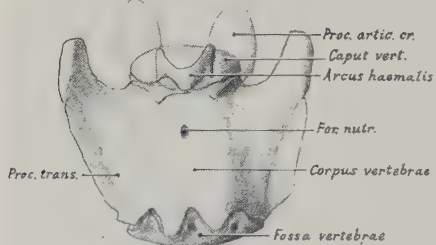
Cranial aspect



Lateral aspect

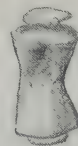
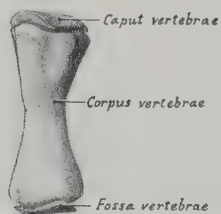


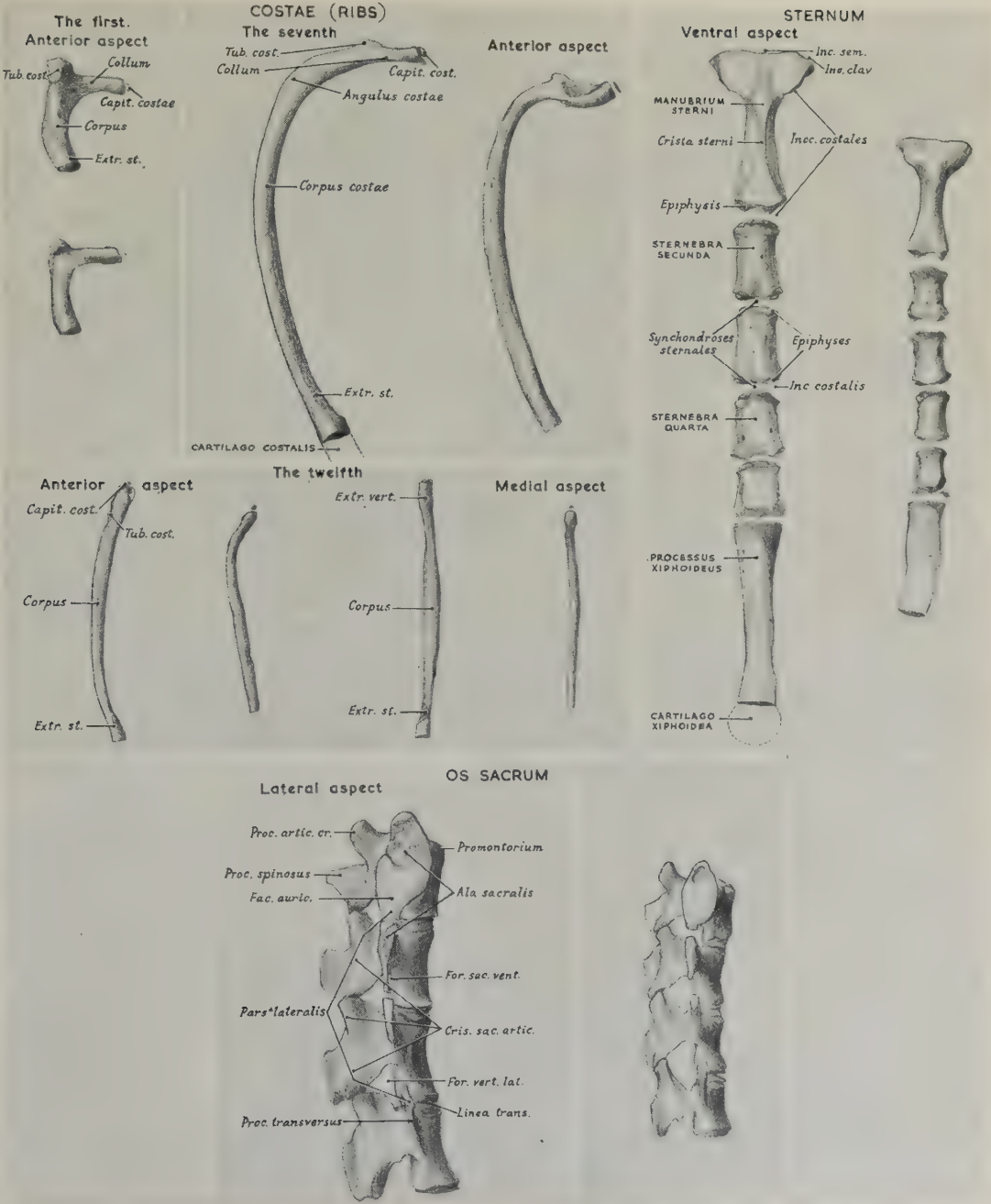
Ventral aspect



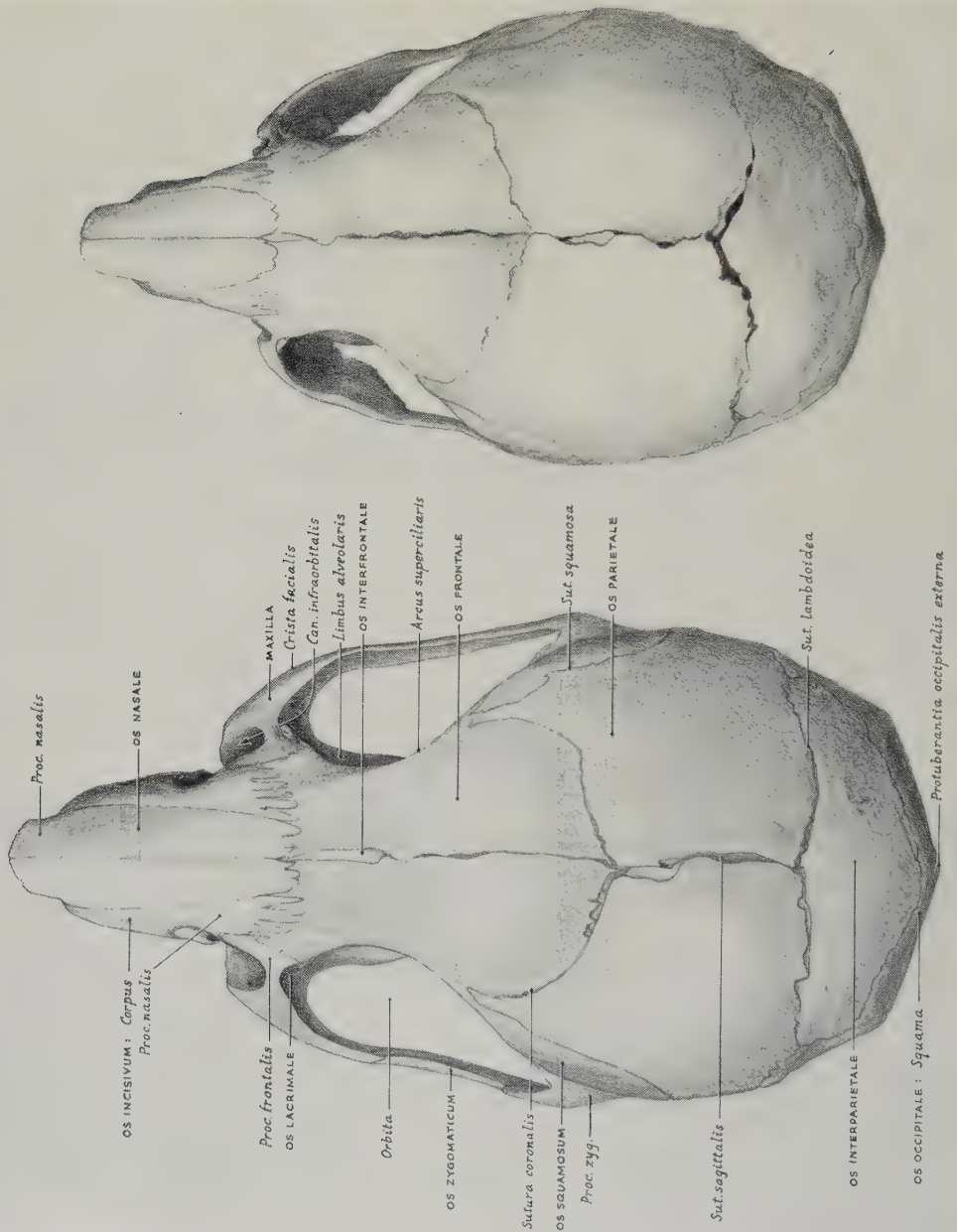
Lateral aspect

12<sup>th</sup> from the last



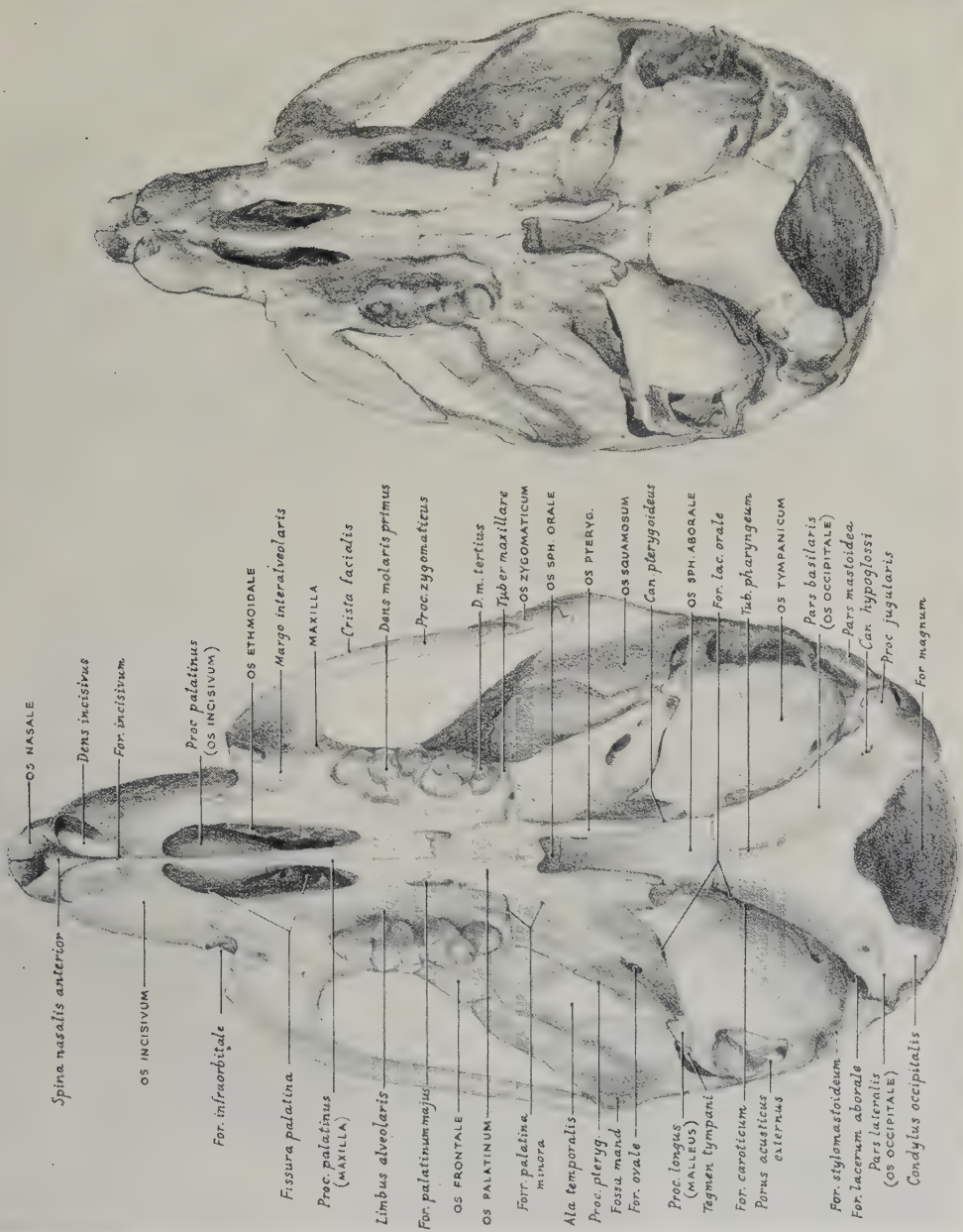


SKULL—Dorsal aspect

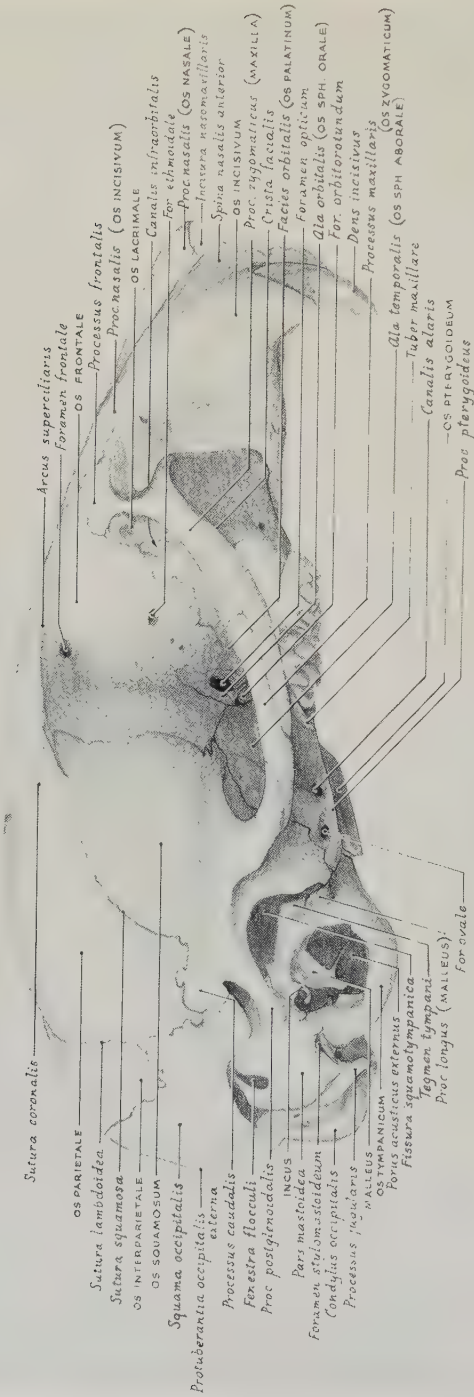




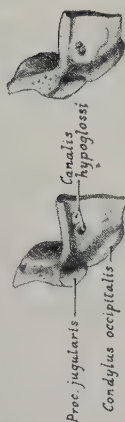
SKULL — Interior aspect



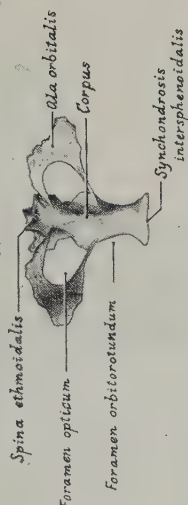
SKULL — Lateral aspect



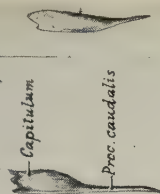
**OS OCCIPITALE—Pars lateralis**  
Naso—ventral aspect



**OS SPHENOIDALE ORALE**  
Cerebral surface



**OS INTERFRONTALE**  
Lateral aspect



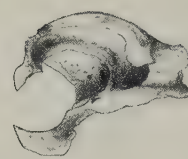
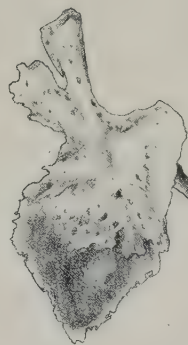
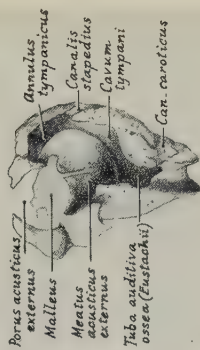
**OS SPHENOIDALE ABORALE**  
Cerebral surface



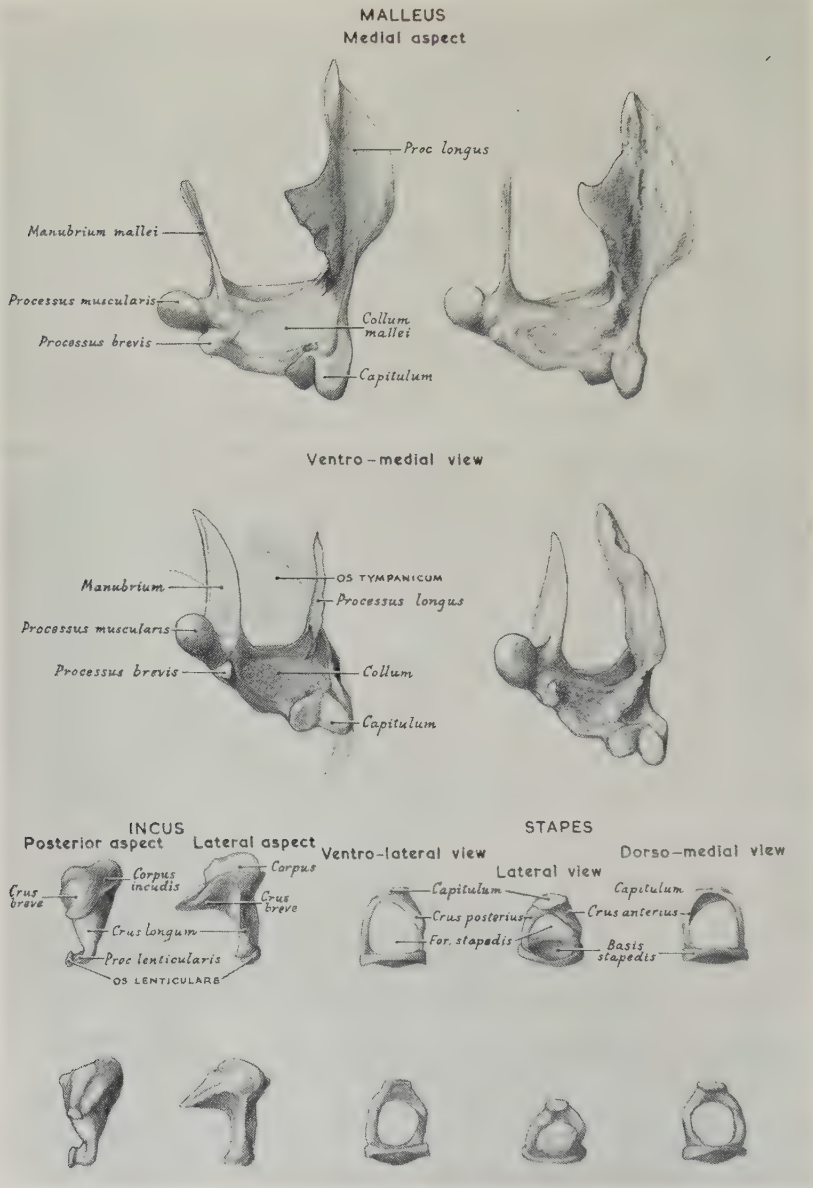
**OS SQUAMOSUM**  
Internal surface  
Sutural overlaps with:—

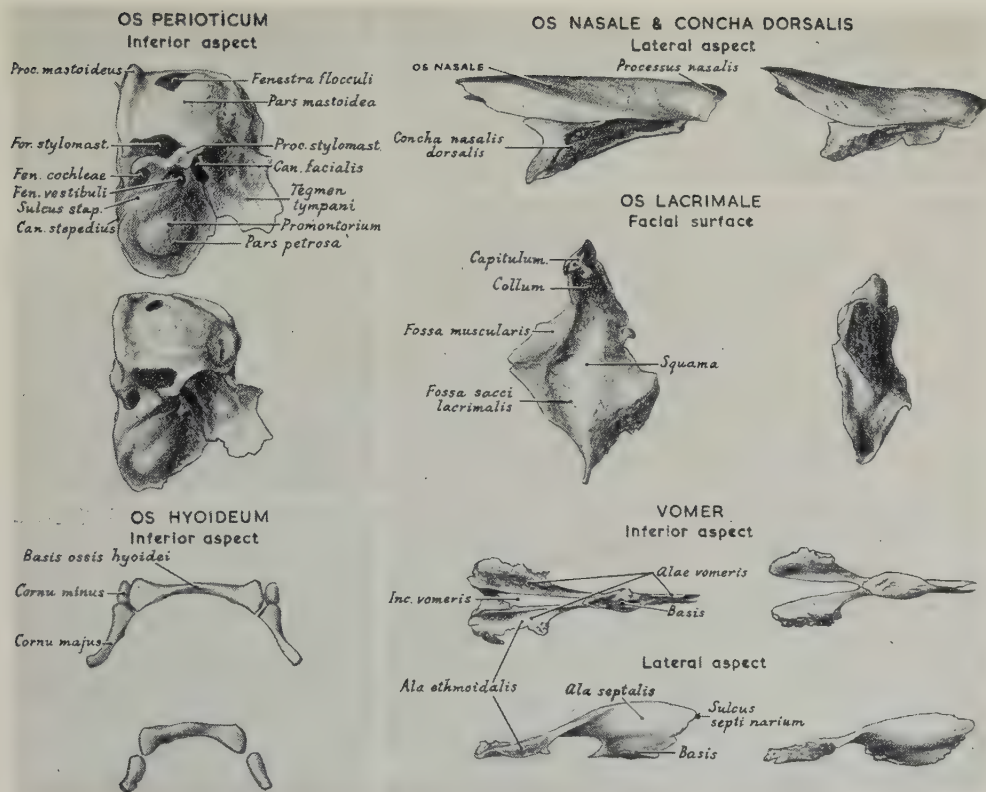


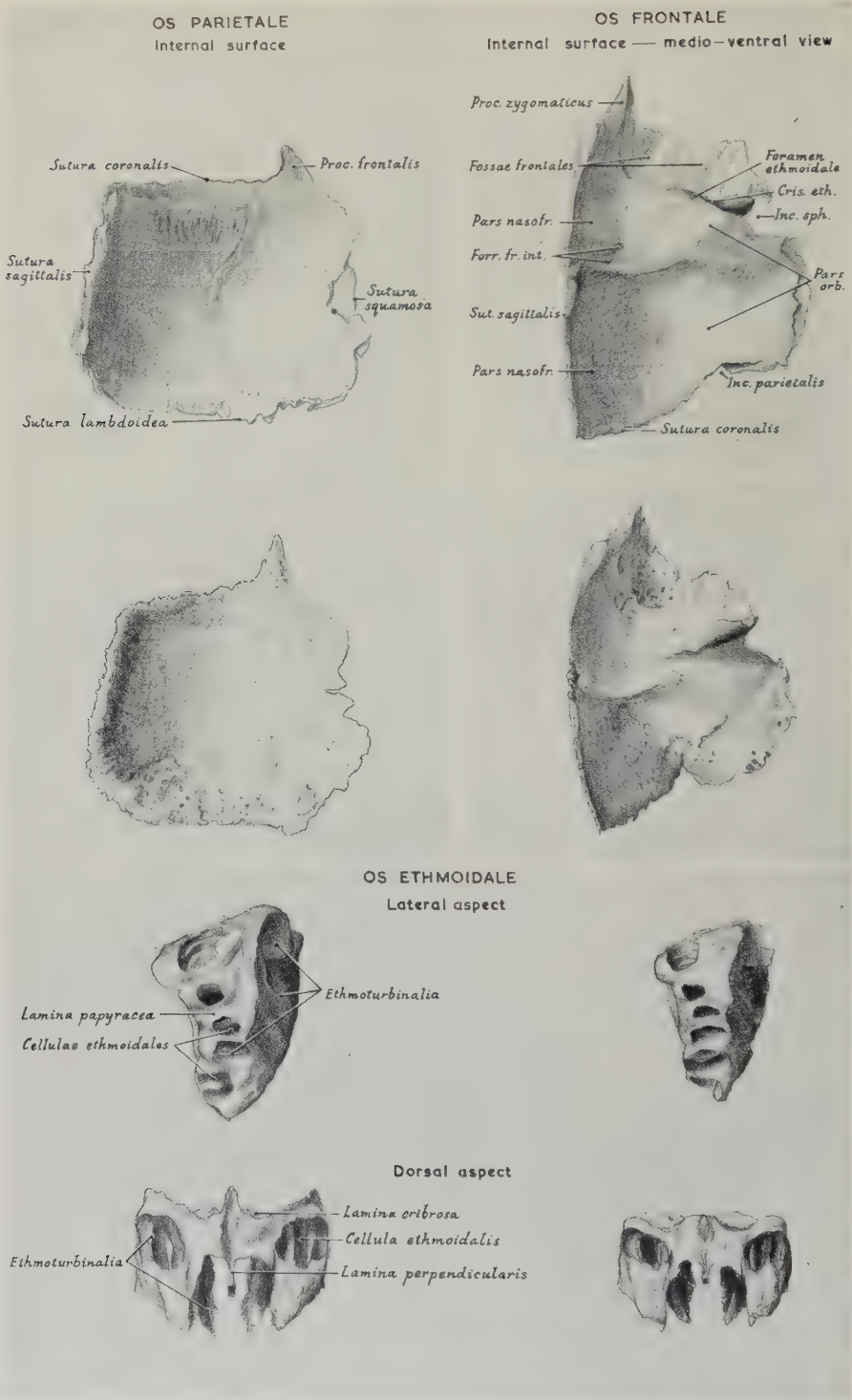
**OS TYMPANICUM**  
Superior aspect



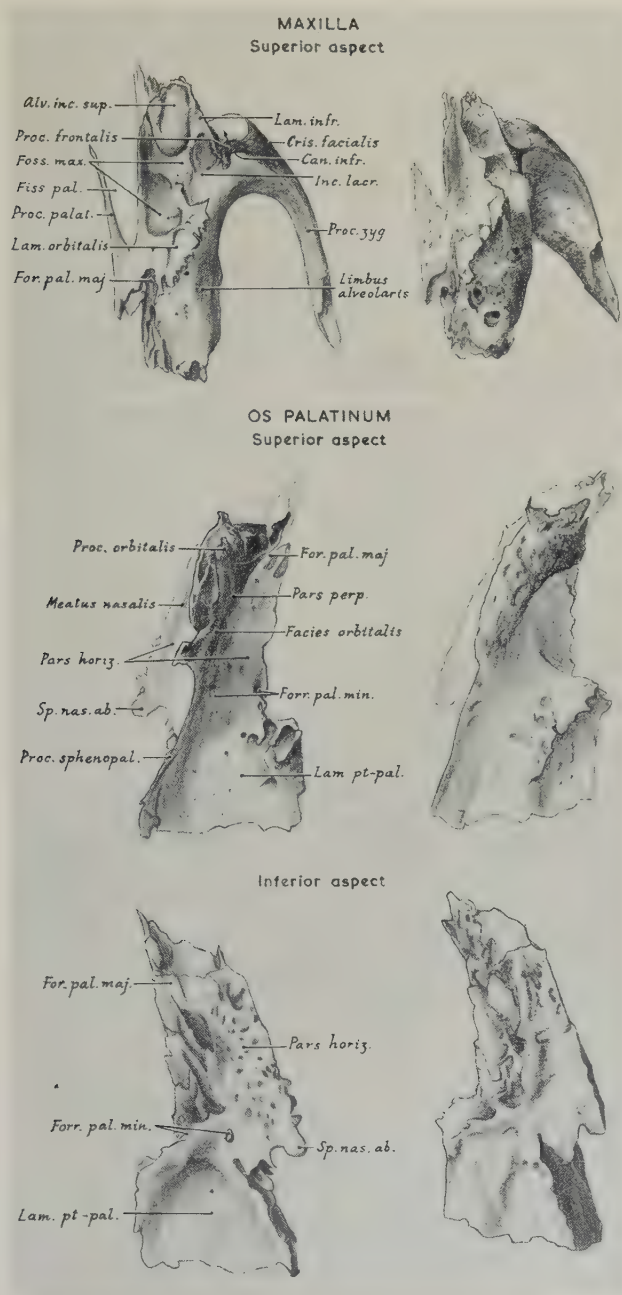






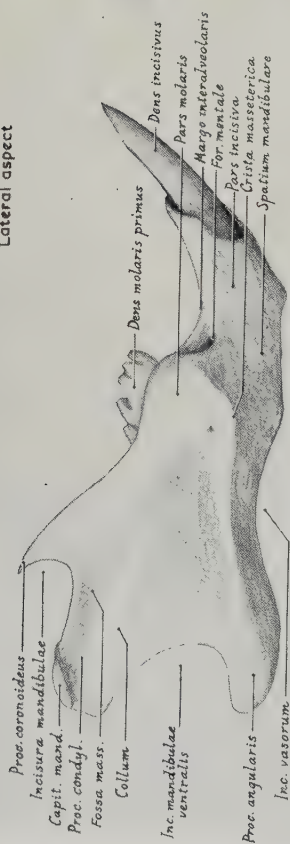






MANDIBULA

Lateral aspect

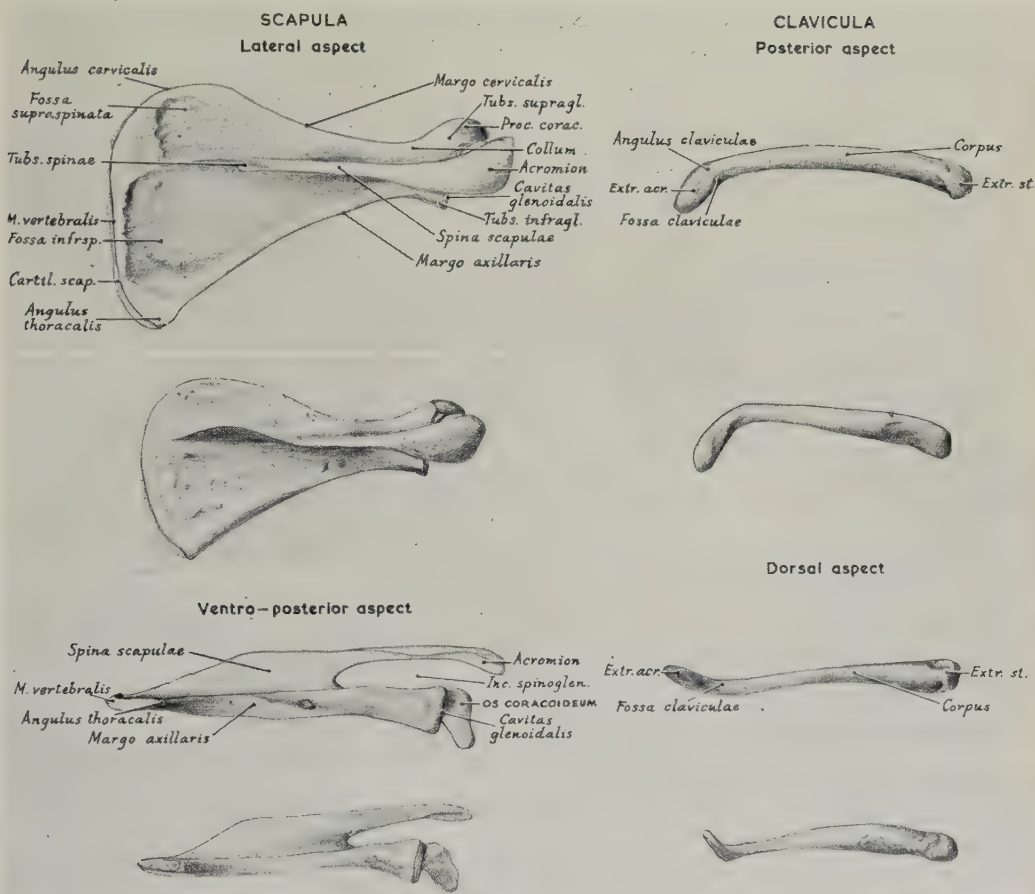


Medial aspect

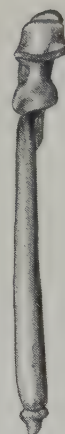
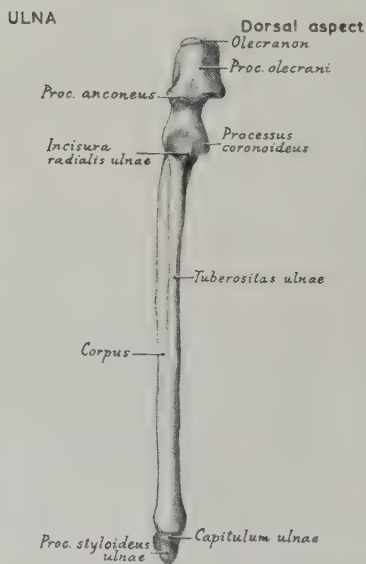
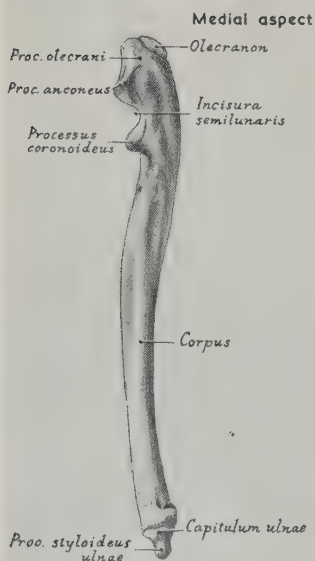
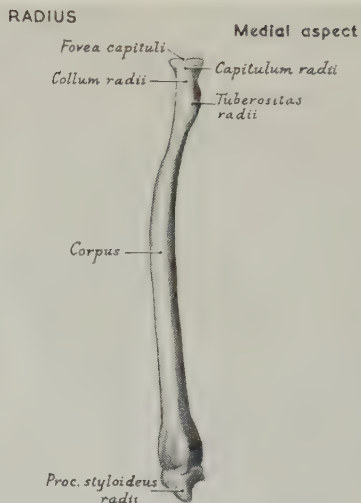
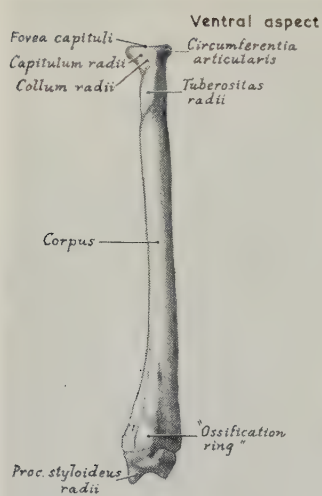


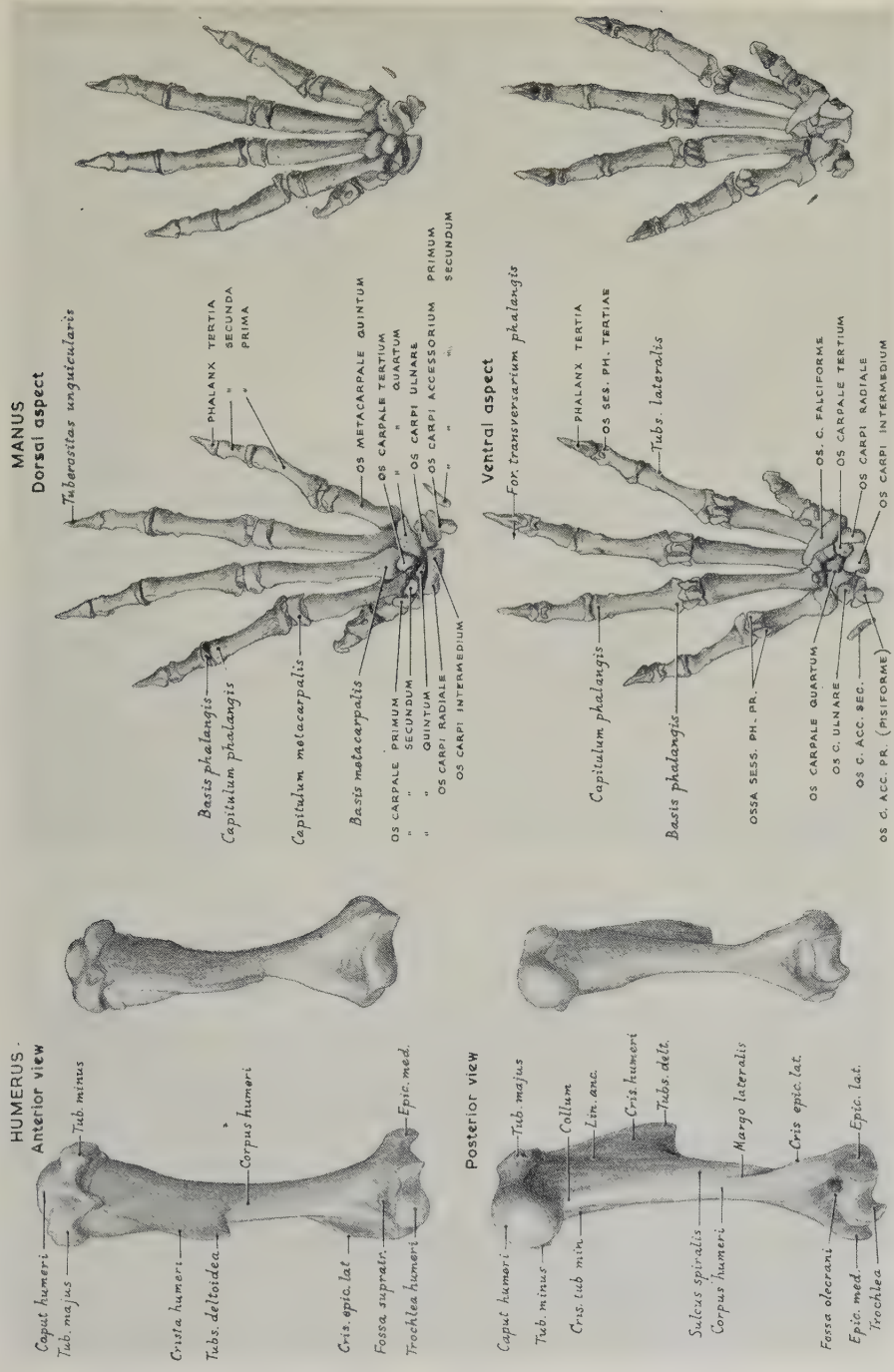
Superior aspect



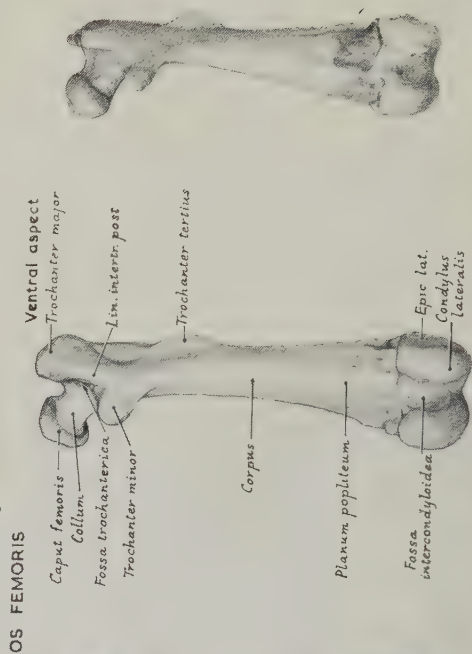
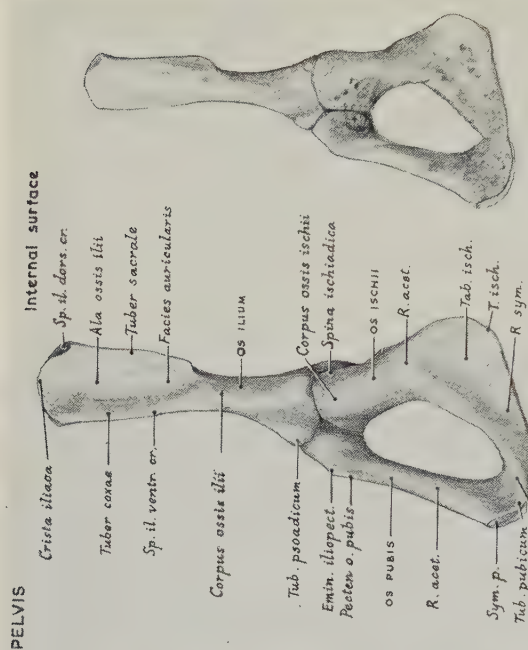




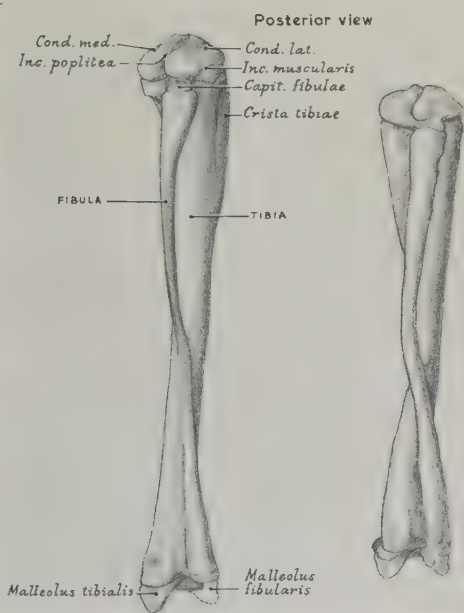
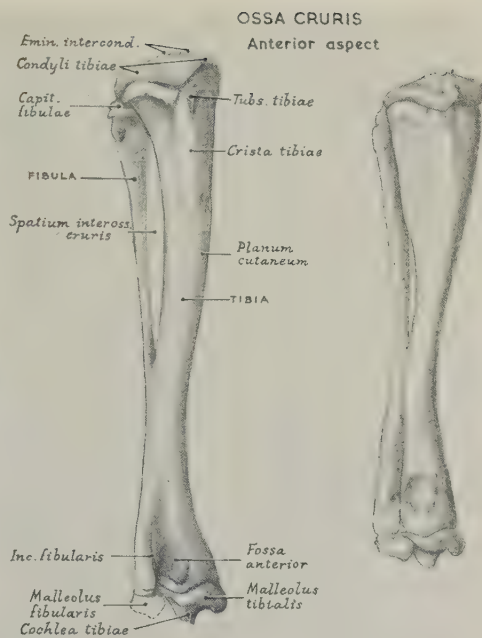
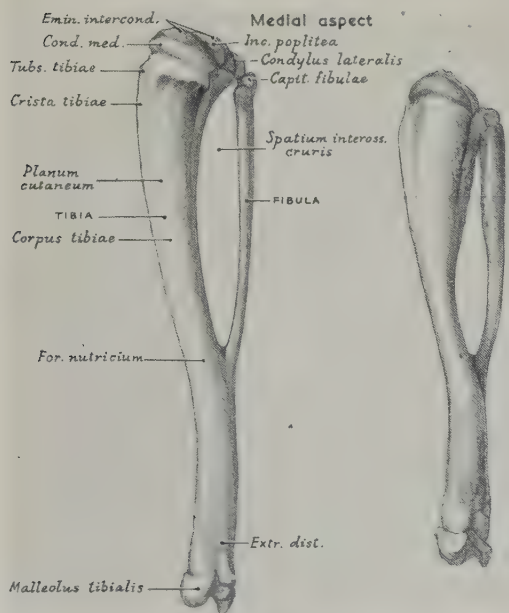
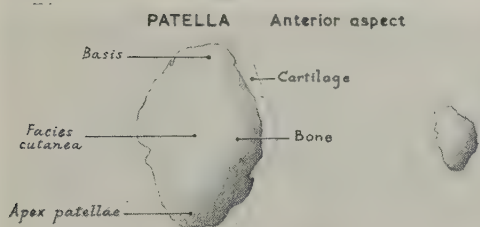
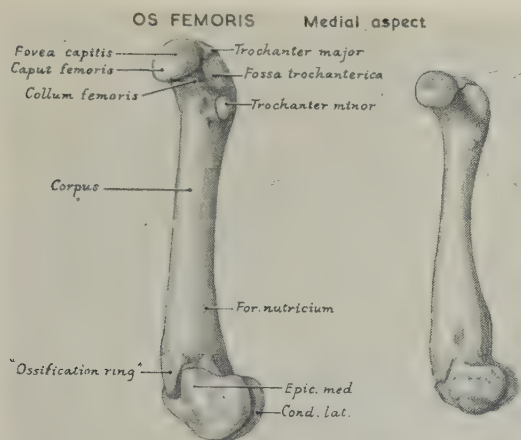




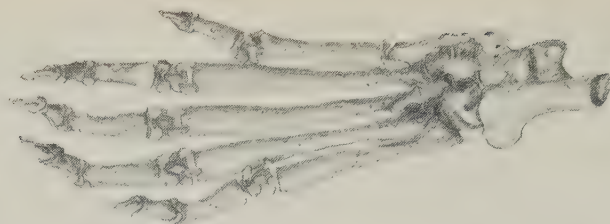
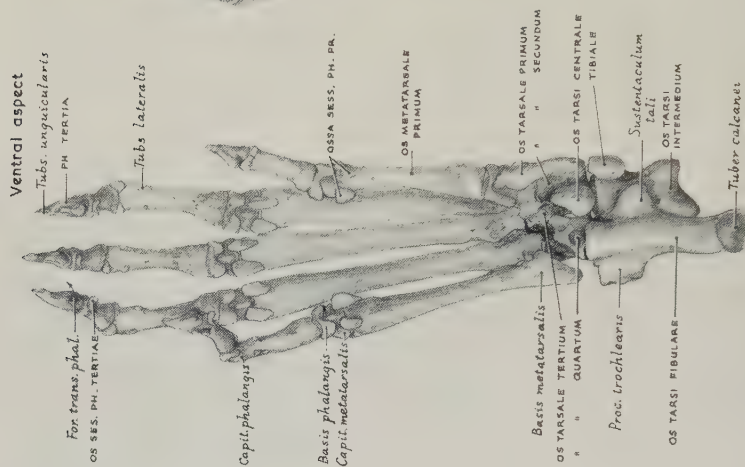
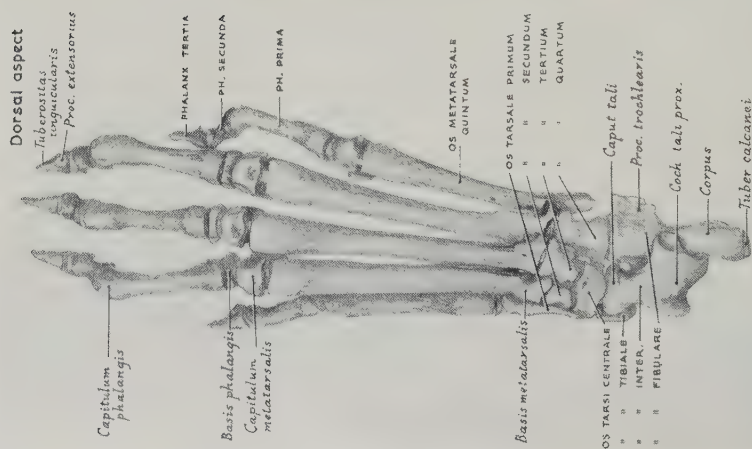
BATEMAN—BONE GROWTH: GREY-LETHAL AND MICROPHTHALMIC MUTANTS OF MOUSE







PES









R. H. BURNE

## IN MEMORIAM

R. H. BURNE

Richard Higgins Burne, M.A. (Oxon.), F.R.S., born in London on 5 April 1868, died on 9 October 1953, a member of the Anatomical Society since 1896. Educated at Winchester and Oriel College, Burne graduated in 1889 and studied natural science under G. B. Howes until appointed (1892) Anatomical Assistant in the Royal College of Surgeons Museum. Therein he served (successively as Assistant Conservator, 1908; Physiological Curator, 1912; and Acting Conservator, 1933) until retirement in 1934, when he was elected Hunterian Trustee. For over forty years, under Charles Stewart and Sir Arthur Keith, Burne curated, catalogued and developed with singular success the immense comparative collections of the Hunterian Museum. For this sustained labour he was admirably equipped by an exceptional personal familiarity with animal structure, an inimitable prosectorial skill, a marked industry and observational faculty, and a natural artistic talent.

He was largely responsible for the published volumes of the Museum's *Catalogue of the Physiological Series* (vol. I, 1900; vol. II, 1902; vol. III, 1907), sharing the authorship of vol. II with Prof. (Sir) Grafton Elliot Smith and Dr W. L. H. Duckworth. Additionally he compiled, revised and illustrated exquisitely the numerous typescript volumes of the unpublished Catalogue, which, combining specimen-descriptions, illustrations, general anatomical information and literature references, constituted a veritable Summa Zoologica. These, with most of Burne's choice and delicate museum preparations, perished in 1941 from enemy action. Upon retirement Burne worked at cetacean anatomy in the British Museum (Natural History) where his preparations are permanently exhibited. In 1939 war interrupted, and, later, increasing blindness terminated, his anatomical activity.

Burne was an invaluable and respected Fellow of the Linnean, Zoological and Malacological Societies, to whose Transactions he contributed, as also to those of the Royal Society, to which he was tardily elected in 1927. Upon the Councils and Committees of these bodies he served continuously, in one capacity or another, for half a century. His relatively few publications did not reflect the catholicity and depth of his zoological knowledge: they concerned molluscan anatomy, vascular arrangements in fishes and particular aspects of mammalian structure.

Reticent, innately courteous, and quietly humorous, Burne manifested an impressive integrity of character. His devotion to scientific truth, zeal in its pursuit and indifference to credit after accomplishment remain an example and an inspiring memory.

In mourning the loss of their distinguished and revered colleague, British anatomists extend to his surviving son and daughter their deepest sympathy.

A. J. E. C.

## OBITUARY NOTICES

We regret to announce the death of W. F. M. Fitzgerald, R.N.V.R., M.B., B.Ch., of University College, Cork on 1 July 1953.

We also regret to announce the death of S. A. Maguire, C.M.G., D.S.O., F.R.C.S., on 10 June 1953.

## REVIEWS

*Experimentelle Histopathologie: ein Einführungskurs.* By H. MEESSEN (Pp. 1-153; 125 illustrations; DM 22.50.) Stuttgart: Georg Thieme Verlag. 1952.

The basic idea behind this little volume is excellent. There are 106 short articles, averaging about  $1\frac{1}{2}$  pages each, with one or more photomicrographs. There is in each case a brief description of an experimental procedure, and of the tissue response to it. This is followed by a few key references which give an introduction to the literature. Thus the first article deals with the effects of vitamin A deficiency, has a photograph of a rat's conjunctiva, and gives as a reference the work of Bicknell & Prescott (1946). Vitamin A overdosage, B<sub>1</sub>, C<sub>1</sub>, D<sub>3</sub> and E deficiencies are then similarly considered. Much of the material would be of great interest from the standpoint of experimental anatomy—e.g. nerve degeneration and regeneration, the effect of castration on the hypophysis, Ascheim-Zondek and similar tests, skin and bone transplants, and so on. This is an interesting and stimulating booklet.

J. M. YOFFEY

*Dental Anatomy.* By MOSES DIAMOND. (Pp. xii + 471; 32 plates + 172 illustrations; \$15.00.) New York: The Macmillan Company. 1952.

Dr Moses Diamond was formerly Professor of Dental Anatomy at the Columbia University College of Physicians and Surgeons and the School of Dental and Oral Surgery. This, the third edition of his book has been posthumously edited by his son Eli. It is a large but rather specialized text-book which deals very fully with the gross anatomy of the human teeth and their occlusal relationships, and less thoroughly with some other important aspects of dental anatomy, such as the embryonic development and microscopic structure of the teeth. There is a short section on the comparative dental morphology of vertebrates, but this is not particularly informative, and its inclusion seems to have been hardly worth while.

Dr Diamond was a pioneer in advocating the practice of tooth-carving (the manufacture of replicas of teeth in wax) as an exercise for dental students and gives detailed directions on how this technique should be carried out. He strongly supports the view that 'knowledge of the detailed descriptions of tooth forms is indispensable to the general dental practitioner', and the most valuable parts of his book are those in which the detailed appearances of the individual human teeth are described. To facilitate such description he has worked out a system of nomenclature for different portions, which he terms lobes, of the teeth; this nomenclature can also be employed in the classification of tooth variations. There are many good photographs of tooth variations which might be of interest to those concerned with the identification of teeth for medico-legal purposes, as well as to the dentist.

A very considerable portion of the book is devoted to the general anatomy of the head and neck and contains an anatomical atlas of 32 full-page plates. Some of these plates, in particular those showing macroscopic sections through the whole head, appear to strike a not altogether satisfactory compromise between the realistic and the diagrammatic styles of illustration. The book ends with an account of the growth of the skull and jaws which is of considerable general interest.

This book is intended as a student text-book and may well be of great value in American schools where the teaching of dental anatomy appears to follow rather different lines from those in England. In this country, where there is probably more integration between medical and dental teaching, dental students, who may also dissect some other part of the



body besides the head and neck, are likely to favour a comprehensive text-book such as Gray's or Cunningham's for their general anatomical studies. The inclusion of so much general anatomy in a book such as that under review seems redundant for the purposes of English students. At the same time the treatment of the developmental and microscopic anatomy of the teeth, and of comparative dental morphology, is hardly adequate for their needs. Dr Diamond's book, however, is certainly a useful addition to the library of the teacher of dental students or the specialist in dental anatomy.

A. D'A. BELLAIRS

## BOOKS RECEIVED

- The Lateral Geniculate Nucleus and Visual Histophysiology.* By GORDON L. WALLS. (Pp. 100; 13 figures in text; \$1.25.) University of California Publications in Physiology, vol. 9, no. 1. Berkeley and Los Angeles: University of California Press. 1953.
- Gynaecological and Obstetrical Anatomy and Functional Histology.* By C. F. V. SMOUT and F. JACOBY. Third edition. (Pp. vii+336; 185 figures; 35s.) London: Edward Arnold & Co. 1953.
- Vitamin C Requirement of Human Adults.* Medical Research Council, Special Report Series No. 280. Compiled by W. BARTLEY, H. A. KREBS and J. R. P. O'BRIEN. (Pp. viii+179; 24 plates; 17s. 6d.) London: H.M.S.O. 1953.
- Evolution and Geography.* By GEORGE GAYLORD SIMPSON. (Pp. 64; 30 figures.) Condon Lectures. Eugene, Oregon: Oregon State System of Higher Education. 1953.
- Medical and Scientific Investigations in the Christie Case.* By FRANCIS E. CAMPS. (Pp. xxiii+224; 47 diagrams and photographs and 6 coloured plates, 30s.) London: Medical Publications Ltd. 1953.
- Human Neuroanatomy.* By OLIVER S. STRONG and ADOLPH ELWYN. Third edition. (Pp. xii+482; 357 figures; 57s. 6d.) London: Baillière Tindall and Cox Ltd. 1953.



# THE AMYGDALOID NUCLEI, HIPPOCAMPUS AND OTHER PARTS OF THE RHINENCEPHALON IN THE PORPOISE (*PHOCAENA PHOCAENA*)

BY A. S. BREATHNACH AND F. GOLDBY

*Department of Anatomy, St Mary's Hospital Medical School, London, W. 2*

## INTRODUCTION

It is well known that the porpoise (*Phocaena phocaena*) is an anosmatic mammal, and that it does not possess an olfactory bulb. One might regard its condition as representing the end results of a phylogenetic removal of the olfactory bulb, and it is clearly of interest to compare these results with those of short-term experimental removal in mammals with a fully functioning olfactory apparatus. They will naturally be sought primarily in the 'rhinencephalon', and in a previous paper (Breathnach, 1953) the condition of the prepyriform cortex, olfactory tubercle ('area désert') and the nuclei of the precommissural region or septum were described. These are all parts of the 'rhinencephalon', but it was found that only the prepyriform cortex and the cortex of the olfactory tubercle showed any obvious deficiency. Both these structures have been shown experimentally to receive fibres direct from the olfactory bulb in osmatic mammals. It was noted that all the nuclei of the precommissural region, which have not been shown to receive such fibres in any mammal which has been adequately investigated, were well developed.

The purpose of the present paper is to extend this survey to the remaining parts of the brain which are commonly included under the term 'rhinencephalon'. The most important of these are the amygdaloid complex of nuclei, the hippocampal formation and the entorhinal cortical area. The cortex of the cingulate gyrus, the habenular nuclei, the mamillary region of the hypothalamus and the anterior thalamic nuclei have also been described as rhinencephalic; they will be considered, but more briefly than the three structures first mentioned.

## MATERIALS AND METHODS

The same material was used as in the previous study (Breathnach, 1953), namely the brains of two adult porpoises (*P. phocaena*), fixed in 10% formalin and cut serially at 20 $\mu$  in celloidin. In the two series the planes of section were approximately at right angles to each other; alternate sections were stained with thionin and by Weil's modification of the Weigert method. The plane of section, in relation to the hemisphere as a whole of the series from which most of the observations and illustrations in this paper were made, is shown in text-fig. 7 of the previous paper.

Similar serial sections from the brains of a sheep, rat and a phalanger (*Trichosurus vulpecula*), and of the human hippocampus were available for comparison.



## OBSERVATIONS

*Amygdaloid complex*

Johnston (1923) divided the nuclear components of the amygdaloid complex into two groups, cortico-medial and baso-lateral, and in general the terminology used for the description of the mammalian amygdala is based on his work. It was applied to the human amygdala by Crosby & Humphrey (1941), and has been found equally suitable for the porpoise. Although the amygdala as a whole is usually included in the rhinencephalon, it should be pointed out that only the cortico-medial group of nuclei have been shown to receive direct connexions from the olfactory bulb (Clark & Meyer, 1947; Meyer & Allison, 1949).

*Baso-lateral amygdaloid nuclei*

These form a prominent cell mass throughout almost the whole extent of the amygdala. The components are:

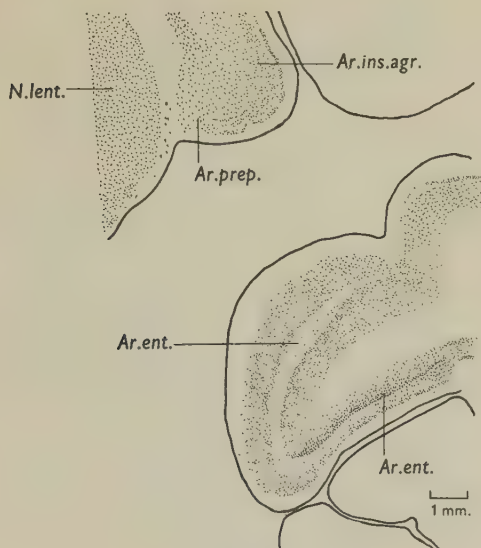
*Lateral nucleus* (Pl. 1, fig. 1; Text-figs. 2-7). This is the most lateral as well as the largest of the amygdaloid nuclei, and is recognizable throughout almost the whole antero-posterior extent of the complex. Its cells vary in size from medium to small and are rounded or fusiform in shape. Although slight regional variations in cell density can be seen, the general impression is of greater homogeneity than in most other amygdaloid nuclei.

Throughout, the nucleus is related laterally to the fibres of the external capsule. Posteriorly it is completely surrounded by fibres and lies in the roof of the lateral ventricle (Text-fig. 6). Except in this region the lateral nucleus has the lateral part of the basal nucleus on its medial side, and although there is some intermingling of cells where the two nuclei are adjacent, the basal nucleus can easily be distinguished since it consists of large multipolar cells, which stain deeply (Pl. 1, fig. 1). The basal and lateral nuclei are not separated by fibres except for a short distance anteriorly. Posteriorly the lateral nucleus comes to lie ventro-lateral to the central nucleus, which separates it from the lentiform nucleus.

It is clear that the lateral nucleus in the porpoise is very similar to the nucleus so named in the fin-whale (Jansen & Jansen, 1953), in man (Crosby & Humphrey, 1941) and in many other mammals (e.g. the cat, Fox, 1940). Its characteristic relationship to the external capsule and to the large-celled part of the basal nucleus as well as its topographical position make its identification reasonably certain.

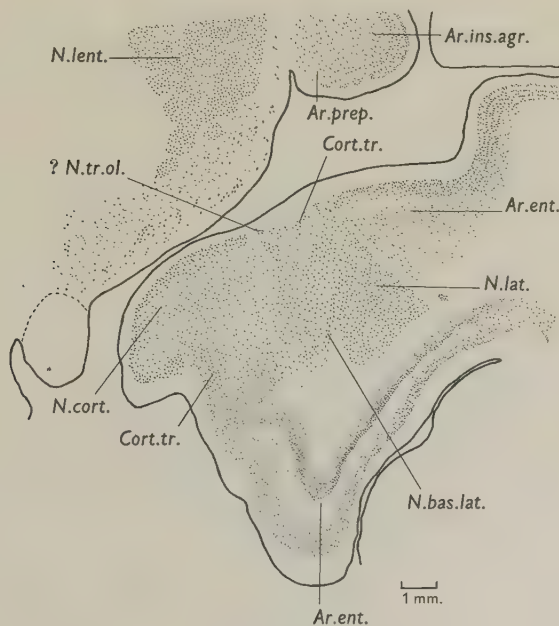
*Basal and accessory basal nuclei.* The basal nucleus as a whole is situated in the medial part of the amygdala (Pl. 1, fig. 1). The lateral or large-celled part is the most conspicuous and clearly defined; it is easily recognized by the size of its cells and their deep staining reaction, and it extends through the whole length of the basal mass (Text-figs. 2-5); posteriorly it is replaced by the central nucleus. Ventrally a few fibres from the stria terminalis enter it, and in doing so separate a small group of the characteristic cells from the ventro-medial angle of the main part of the nucleus (Text-fig. 4).

The medial part of the basal nucleus and the accessory basal nucleus are more difficult to define. They lie in the anterior third of the amygdala (Text-fig. 3; Pl. 1, fig. 1) deep to the cortical nucleus and the cortico-amygdaloid transition area, and

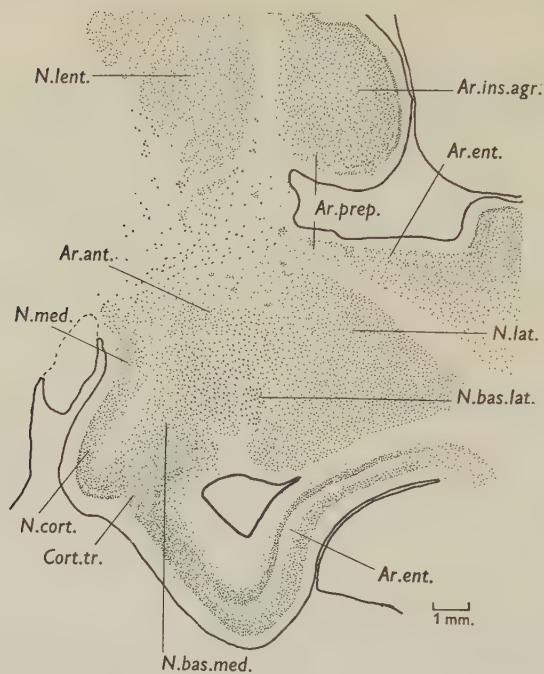


Text-fig. 1.

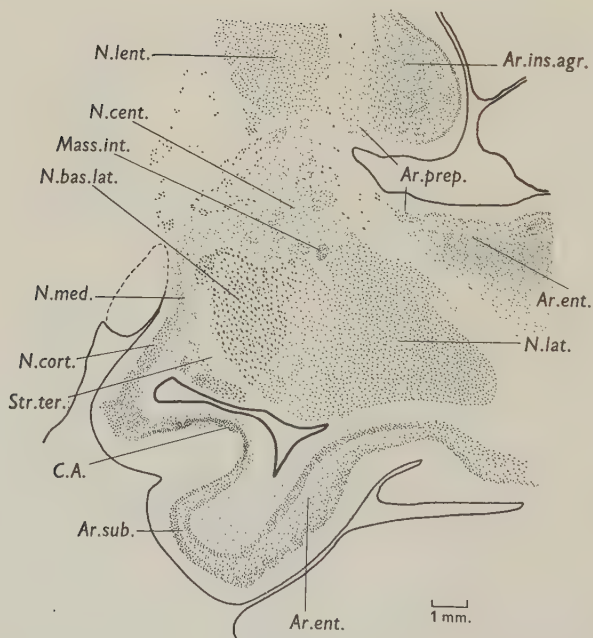
Text-figs. 1-7. These figures are drawings of a series of sections cut in the coronal plane and stained with thionin, passing cranio-caudally from the tip of the temporal pole to the caudal extremity of the amygdaloid complex. Parts of the hippocampus and adjacent cortical areas and the corpus striatum are shown in addition to the amygdaloid nuclei. For the list of abbreviations see p. 291.



Text-fig. 2.

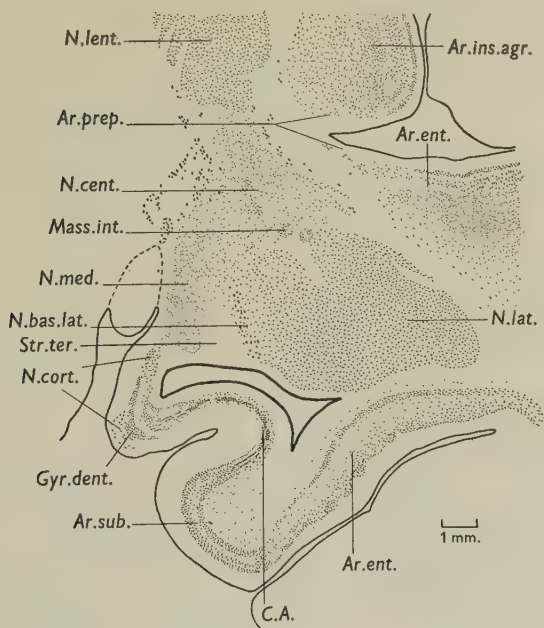


Text-fig. 3.

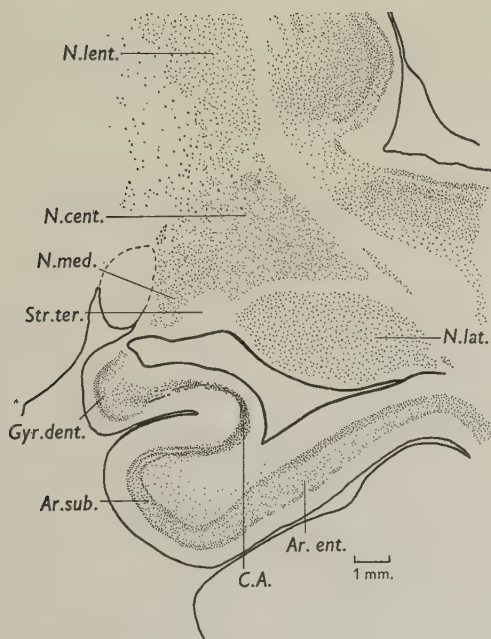


Text-fig. 4.



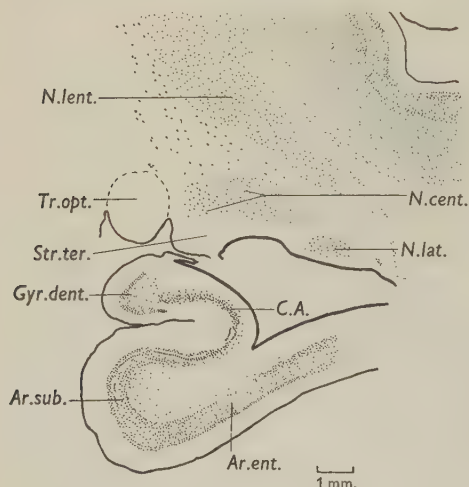


Text-fig. 5.

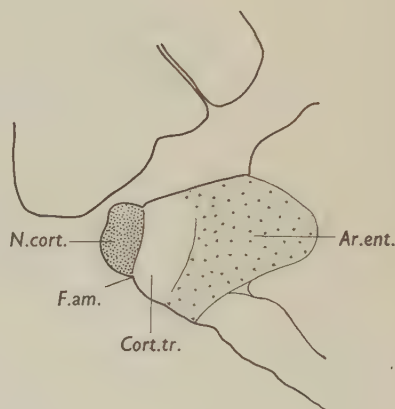


Text-fig. 6.

are closely related to fibres which appear to be derived mainly from the stria terminalis system. The medial part of the basal nucleus is divided in man into superficial and deep parts (Crosby & Humphrey, 1941). Indications of a similar subdivision can be seen in the porpoise; small cells, immediately deep to the cortico-amygdaloid transition area, may represent the superficial part and larger cells, which stain poorly, situated just ventral to the lateral part of the basal nucleus, a deep part. The significance of these very ill-defined subdivisions is doubtful.



Text-fig. 7.



Text-fig. 8.

Text-fig. 8. A diagram showing the cranial aspect of the medial side of the left temporal pole; the cut surface of the frontal lobe is shown in the upper part of the diagram separated by the Sylvian fossa from the temporal pole. Parts of the surface extent of the cortical amygdaloid nucleus, cortico-amygdaloid transition area and the entorhinal cortical area are indicated.

The accessory basal nucleus is probably represented by a more or less discrete group of medium-sized cells, immediately deep to the cortical nucleus (Pl. 1, fig. 1) and separated from it by a layer of fibres.

*Intercalated cell-masses.* These are discrete islands of small cells which have been described in the amygdala of most mammals between the main nuclei. In the porpoise they are found in association with the lateral nucleus, mostly on its dorsal aspect between it and the central nucleus (Text-figs. 4, 5). An occasional group may be found between the lateral and basal nuclei. Their appearance is similar to that described by Jansen & Jansen (1953) for the fin-whale.

The features of the basal nuclei of the porpoise are clearly very much the same as those described for the region similarly named in other mammals. This applies particularly to the large-celled or lateral basal nucleus, which is easily recognized and defined, and appears to be one of the most striking features of the amygdala in most, perhaps in all mammals. Of the medial part and the accessory basal nucleus it can be said only that they constitute an ill-defined mass of cells in which regional variations in cell size and density can be recognized; while the mass as a whole is probably comparable from one mammal to another, it is uncertain if the regional

variations form a definite enough pattern for more detailed comparisons to be valid. It may be noted that Jansen & Jansen (1953) were unable to distinguish an accessory basal nucleus in the fin-whale, but apart from this, their account corresponds very closely with that given above.

### *Cortico-medial amygdaloid nuclei*

In general, these nuclei occupy a position close to the surface and dorso-medial to the baso-lateral group. The main constituents are the cortical, medial and central nuclei and the nucleus of the lateral olfactory tract. In the anterior part of the amygdala the medial and central nuclei are replaced by a region in which there is no differentiation of nuclear groups, but where numerous small cells are present scattered irregularly among bundles of fibres (Text-fig. 3). This region, which lies dorsal to the baso-lateral group of nuclei and immediately ventral to the putamen, is the 'anterior amygdaloid area' of Crosby & Humphrey (1941) and of other authors. There is also a cortico-amygdaloid transition area.

*Cortical nucleus.* This nucleus forms a small elevation on the medial side of the temporal lobe (Text-fig. 8) and is surrounded by the shallow amygdaloid fissure. Its structure (Pl. 1, fig. 1) is very similar to that of prepyriform cortex. There is a superficial or molecular layer, free of cells, but containing fine myelinated fibres. This is succeeded by a narrow intermediate layer of fairly closely packed pyramidal cells which passes without any defined boundary into a broader third layer containing cells similar in type but more scattered in their arrangement. Anteriorly there is no boundary between these deeper cells and the adjacent medial basal nucleus; posteriorly a layer of fibres, probably part of the stria terminalis system, intervenes. On the surface posteriorly the cortical nucleus is replaced by the small cells of the dentate gyrus (Text-figs. 4-6).

The cortico-amygdaloid transition area (Pl. 1, fig. 1; Text-figs. 2, 3 and 8) lies between the cortical nucleus and the entorhinal area. Its cells, which are mainly pyramidal or fusiform in shape, are somewhat concentrated towards the surface from which they are separated by a molecular layer; they are not clearly separated from the cells of the underlying medial basal nucleus where the cells become smaller.

In sections through the anterior part of the amygdala a few scattered cells are found closely associated with the dorso-lateral edge of the cortical nucleus (Text-fig. 2), a situation similar to that occupied by the nucleus of the lateral olfactory tract in Primates (Crosby & Humphrey, 1941; Lauer, 1945). These cells appear to be the only possible representative of this nucleus in the porpoise, and if so, it is so poorly developed as to be barely recognizable. It is of interest to note that Jansen & Jansen (1953) found it quite well defined in the fin-whale.

The correspondence between the cortical nucleus in the porpoise and the same nucleus as described by Crosby & Humphrey (1941) in man, by Lauer (1945) in the macaque, Fox (1940) in the cat, and by other authors in other mammals, is very close. It is represented by Brockhaus's (1938) periamygdala and Rose's (1927) areas *Pam*, 2 and 3. In the porpoise it is well differentiated, and probably rather better defined than in Primates. No indication could be found of a division into dorsal and ventral parts as described by Jansen & Jansen (1953) in the fin-whale, nor was the



more superficial of its two cellular layers lacking in any situation, as these authors found.

*Medial nucleus.* This is found on the surface between the optic tract and the basal nucleus (Text-figs. 3-6). Ventrally it is directly related to the cortical nucleus, and dorsally extends laterally over the basal nucleus to come into relation with the central nucleus. Anteriorly it gradually becomes smaller, cell density falls off and it blends with the anterior amygdaloid area. Posteriorly it increases in size, cell density increases and it disappears by merging with the central nucleus. The cells are predominantly small in type, smaller, for instance, than those of the lateral nucleus, but, here and there, especially towards the posterior end, scattered cells of a larger size are encountered.

*Central nucleus* (Text-figs. 4-7). This is probably the least well defined of any of the amygdaloid nuclei. It consists of cells, mostly of medium size, generally slightly larger than those of the medial nucleus, irregularly scattered among fibre bundles. Throughout, it lies ventral to the lentiform nucleus from which it is not clearly separated although the cells of the latter are somewhat larger. It merges with the medial nucleus, and anteriorly, without any obvious boundary, the anterior amygdaloid area. Posteriorly it is continuous with the bed nucleus of the stria terminalis, and in this region there is a concentration of rather large cells towards its medial side. These may correspond with the large-celled part of the central nucleus described by Brodal (1947*b*) and Fox (1940) for the rat and cat respectively.

It seems clear that the whole region of the medial and central nuclei and the anterior amygdaloid area could be described as a field containing diffusely scattered cells in which ill-defined regional variations in cell density and size occur, and that it is a continuation forwards of the bed nucleus of the stria terminalis. So far as the medial and central nuclei are concerned, this was indeed Johnston's (1923) suggestion when he described these nuclei as an enlargement of the bed nucleus of the stria. The region is very similar in all mammals, including the porpoise; one gets the impression in this animal that the part called 'medial nucleus' contains fewer cells than in most macrosmatic mammals, especially anteriorly, and that the 'central nucleus' is perhaps larger than in Primates (cf. figures in Crosby & Humphrey, 1941; and Lauer, 1945), but in the absence of clearly defined boundaries these comparisons can have little significance.

*Stria terminalis.* This consists of finely myelinated fibres, accompanied by the cells of its bed nucleus, which can be followed in the usual position around the lentiform nucleus. These fibres are closely related to the basal, central and medial amygdaloid nuclei, but precise details of connexions within the amygdala cannot be established in material of the kind available. The system does not appear to be significantly different from what can be seen in similar material from the sheep.

One may sum up this account of the amygdala in the porpoise by stating that its nuclear configuration conforms to the general mammalian plan, all nuclei being present with the probable exception of the nucleus of the lateral olfactory tract. Its differentiation, and particularly that of the baso-lateral complex, resembles very closely what is found in the Primates (Crosby & Humphrey, 1941; Lauer, 1945). We have found a closely similar pattern in the sheep, and Fox's description (1940) shows that the condition in the cat is also much the same. It is clear that the

amygdala of the fin-whale (Jansen & Jansen, 1953) does not differ significantly from this general pattern, which is indeed remarkably constant in all the animals mentioned.

In relatively smooth-brained mammals such as the rat (Gurdjian, 1928; Brodal, 1947*b*), bat (Humphrey, 1936), shrew (Crosby & Humphrey, 1944), opossum (Johnston, 1923), rabbit (Young, 1936), the position is not quite the same. Most authors have in fact distinguished and named the same nuclei as we have described in the porpoise, and Johnston, who introduced this terminology, based it on the condition he found in *Didelphys*. One finds, however, that Gurdjian's and Brodal's interpretations in the rat differ significantly from one another; that Young (in the rabbit) finds the lateral nucleus divisible into an anterior large-celled and a posterior small-celled part, while in the rat the condition is reversed (Brodal, 1947*b*). The figures published by Crosby & Humphrey (1944) for the shrew show very little in the way of well-defined nuclei, and suggest that the material on which they are based might reasonably be open to more than one interpretation. We have made no detailed study of the amygdala in mammals of this kind, but a preliminary examination of serial sections of the rat's brain and of the brain of the phalanger, *Trichosurus*, show that the difficulties of interpretation in terms of the subdivisions of the amygdala usually made, are real, and very much greater than in mammals with larger and more highly convoluted brains.

Main interest, of course, centres on the question whether the absence of olfactory bulbs and tracts in the porpoise can be related to any morphological differences between the amygdala in this animal and in mammals in which the olfactory apparatus is well developed. The virtual absence of the nucleus of the lateral olfactory tract is clearly the most obvious of these differences. All other amygdaloid nuclei are present in the porpoise, and if the loss of olfactory connexions has had any effect on their morphology it can be only in altering the degree of development of some or all of them relative to the size of the brain as a whole or to each other.

Morphological studies, such as those of Crosby & Humphrey (1941) and Lauer (1945) indicate that in microsmatic mammals like the Primates, where the non-olfactory cortex is very extensive, the nuclei of the cortico-medial group are much smaller than those of the baso-lateral, a disproportion which is said to be less evident in the brain of macrosmatic mammals such as the rat. It is implied that a progressive increase in this disproportion accompanies the development of the microsmatic condition, and, *a fortiori*, it should be the more evident in an anosmatic brain. While simple inspection of sections may give this impression it is difficult to make allowance for the fact that some nuclei may appear only or mainly in one part of a series, where other nuclei may not be visible at all. Moreover, quite small differences in the orientation of the amygdala (or of the plane of section) may alter appearances very greatly. We ourselves felt that in the sheep, an animal with a well-developed olfactory apparatus, the cortico-medial nuclei were rather better developed and proportionately larger than in the porpoise, although the difference was not very striking. We found, however, that in the bat, with a well-developed olfactory bulb, and with a poor development of the non-olfactory cortex, Humphrey (1936) described the baso-lateral complex as 'relatively very much larger than the cortico-medial'.

For these reasons it seemed desirable to supplement visual impressions as far as possible with objective measurement, and we have chosen the sheep's brain for comparison with that of the porpoise, since the general similarity in the differentiation of the amygdaloid nuclei is so marked that there is no difficulty in identifying the same nuclei in the two animals. It would be much more difficult to institute valid comparisons with the smaller smooth-brained mammals mentioned above, although many of these show the macrosmatic character to a higher degree than the sheep.

On the complete series available, the antero-posterior length, maximum width and total volume of the amygdala have been measured, and also the volume of the baso-lateral group of nuclei. All measurements of course apply to the fixed and embedded brain. The maximum width in the porpoise was taken from the surface of the cortical nucleus to the most lateral point on the lateral nucleus; owing to a difference of the orientation of the complex as a whole, the corresponding measurement in the sheep is almost vertical. Volumes were estimated by planimetry, using the method of Dornfeld, Slater and Scheffé (1942).

*Dimensions of amygdala*

	Antero-posterior length (mm.)	Maximum width (mm.)	Total volume (mm. <sup>3</sup> )	Volume of baso-lateral nuclei (mm. <sup>3</sup> )
Porpoise	12	12	2590	1480 (57 %)
Sheep	6	10	690	380 (55 %)

It will be seen that the total volume of the amygdala in the porpoise is about 4 times that of the sheep; the total brain weight (including brain stem and cerebellum) of the porpoise was about 500 g., and the sheep's brain varies from about 100 to 120 g. (the exact weight of the brain from which the sections were made was not known); that is to say the total brain weight of the porpoise is from 4 to 5 times that of the sheep, and one may say that the increase in volume of the amygdala in the porpoise is about proportionate to the total increase in brain weight. It appears, therefore, that the loss of olfactory connexions has had no great effect on the total size of the amygdala, compared to the size of the brain as a whole. Some figures given by Jansen & Jansen (1953) for the fin-whale are also relevant to this question. They give the maximum length and width of the amygdala as 25 and 20 mm. respectively. Jansen (1952) gives the total brain weight in this animal as 6850 g. The linear dimensions of the amygdala in the fin-whale are therefore about twice those in the porpoise, suggesting that the volume would be increased about 8 times. Total brain weight in the fin-whale is nearly 14 times that of the porpoise, so that the increase in size of the amygdala is considerably less than that of the brain as a whole, in spite of the presence of olfactory connexions in the fin-whale.

When the relative volumes of baso-lateral and cortico-medial nuclear groups are considered, it is found that there is practically no difference between the porpoise and the sheep. In both, the volume of the baso-lateral is greater than that of the cortico-medial group, the actual figure being between 50 and 60 % of the total volume of the amygdala. Since the sheep is macrosmatic and the porpoise anosmatic this is a surprising finding, and throws doubt on the idea that, in large-brained mammals at least, the presence or absence of olfactory tract connexions has any great effect on the relative sizes of these different parts of the amygdala.



It must of course be admitted that the volume measurements we have given are very approximate. The fact that so much of the amygdala is made up of 'nuclei' with very ill-defined boundaries, introduces a serious uncertainty into any measurements of area made in transverse sections, and it is on these measurements that the estimates of volume are based. Moreover, the boundary between the amygdala as a whole and the lentiform nucleus is by no means clear cut. Small differences in volume, which might have important functional significance, obviously could not be detected in material of this kind. One can only say that previous estimates, based simply on the inspection of sections, are open to at least as much uncertainty and are more likely to be affected by subjective bias. From our own findings we feel that it is fair to conclude that so far, no correlation between the relative size of the cortico-medial group of nuclei as a whole and the abundance or absence of olfactory tract connexions has been established, at least in mammals such as the sheep and porpoise with relatively large brains.

#### THE HIPPOCAMPAL FORMATION

Though traditionally a part of the 'rhinencephalon', the hippocampus is now regarded as having only a remote association with olfaction, if indeed it has any at all (Brodal, 1947*a*; Kaada, 1951; Allison, 1953). One of the subsidiary arguments advanced in support of this view is the fact that the anosmatic cetacea possess a recognizable hippocampus. It has also been noted, however, that the cetacean hippocampus is small, a fact accepted by some authors (e.g. Addison, 1915) as suggestive evidence that the larger hippocampus of other mammals is at least partly concerned with olfaction. For these reasons it is clearly desirable to investigate the structure of the cetacean hippocampus in greater detail than has been done previously, and, if possible, to obtain estimates of its absolute and relative size.

In the account which follows the parts included under the term hippocampal formation are the dentate gyrus, cornu ammonis, and the subicular cortex, together with the extension of these structures above the corpus callosum as the induseum, which ends in the rudimentary anterior hippocampal cortex above the septum. There are also the associated fibre systems of which the fornix is the most important.

#### *General form*

The same parts and general relations are found as in other mammals. A typical dentate gyrus and cornu ammonis are found on the medial side of the temporal lobe (Pl. 1, fig. 4) extending posteriorly from the region of the cortical amygdaloid nucleus. As the splenium of the corpus callosum is approached these structures become considerably smaller, and the differentiation between them is lost (Pl. 1, fig. 5). There is no subcallosal 'hippocampal flexure' (Elliot Smith, 1897), and the formation can be followed directly round the splenium into continuity with a well-marked induseum (Pl. 1, fig. 6).

#### *Cytoarchitecture*

*Dentate gyrus.* Where this is clearly differentiated from the cornu ammonis transverse sections show a typical curved lamina of 'granule' cells (Pl. 1, fig. 4), superficial to which is a molecular layer containing a very few scattered cells. The

granule cells stain rather lightly in our preparations, and are less densely packed than in most mammals, especially those in the dorsal limb of the curve. At some levels, looser packing of the more deeply situated cells suggests a division into two laminae. Deep to the granular lamina is a region containing sparsely scattered cells of varying type; this region is included in the dentate gyrus as the polymorph layer by some authors, while others regard it as belonging in whole (Rose, 1926) or in part (Lorente de No, 1934) to the cornu ammonis. A typical dentate gyrus of this form extends over only a short distance. Anteriorly the granular layer loses its curvature, becomes orientated vertically (Text-fig. 5) and is replaced by the cortical amygdaloid nucleus (Text-fig. 4). Posteriorly the orientation becomes horizontal, and the granular lamina is reduced to a slender tail of small cells directly continuous with the small cornu ammonis (Pl. 1, fig. 5). Some distance anterior to the splenium even this vestige of the dentate gyrus is lost and in the induseum behind the splenium and above the corpus callosum nothing can be seen to represent it.

*Cornu ammonis.* On the basis of preparations in which fibres as well as cells were stained, six or more layers have been distinguished in the cornu ammonis (Lorente de No, 1934, and others). In our material only the main cellular layer, the stratum pyramidale, can be investigated in detail. The stratum radiatum, lacunosum and moleculare superficial to it and the stratum oriens on its deep aspect, require silver impregnation or Golgi material for their adequate investigation. Our Weigert preparations were not suitable, although they showed the alveus layer of fibres clearly enough.

The stratum pyramidale (Pl. 1, fig. 4) consists of large cells which are rather less frankly pyramidal in shape than in other mammals, being somewhat rounded. They have the appearance of being less closely packed than usual and the superficial and deep surfaces of the stratum are not very clearly defined. There is a noticeable scattering of the cells into the stratum oriens. It must be admitted, however, that direct comparison with sections of the human hippocampus does not show any conspicuous differences in these respects.

The stratum pyramidale extends from the concavity of the dentate gyrus to the subiculum, but the subicular border is indefinite. In Pl. 1, fig. 4, we have placed it at the point marked with an arrow, where a frankly bilaminar character becomes apparent, a point which can be recognized in all sections through the fully differentiated part of the hippocampus. Within the cornu ammonis defined in this way the following fields can be differentiated:

(a) The scattered cells within the hilum of the dentate gyrus. In the porpoise there are considerably fewer than in most mammals, and it may be that they should be classed as a polymorph layer of the dentate gyrus rather than with the cornu ammonis (see above).

(b) A fairly broad, ill-defined lamina of cells, many of which appear to be bipolar in type. These cells are more loosely packed than those in other parts of the cornu ammonis.

(c) A large area occupying the main convexity of the cornu in which cells of the same type form a more closely packed, thinner and better defined lamina than in (b). In a few sections a break can be seen in this lamina (X, Pl. 1, fig. 4) separating it from the next area.

(d) A smaller area in which the cell lamina again broadens and begins to show signs of a bilaminar character. As stated above, the point where the bilaminar character becomes definite has been taken as the boundary of the cornu ammonis. It is quite possible however that the field (d) corresponds with the prosubiculum of Lorente de No (1934), but the criteria available in Nissl preparations alone cannot decide this point.

This description applies only to that short length of hippocampus in which the dentate gyrus and cornu ammonis show the characteristic form illustrated in Pl. 1, fig. 4. Posteriorly, and before the level of the splenium is reached, the stratum pyramidale becomes much less extensive (Pl. 1, fig. 5). It begins beneath the fimbria, adjacent to the few small cells which represent the granular stratum of the dentate gyrus. From this point, it can be followed round the convexity of the cornu, gradually increasing in depth, and showing more and more evidence of subdivision until a fully laminated cortex is reached. Presumably part of the region of transition is subicular, but the absence of any abrupt change makes the placing of precise boundaries impossible, and any attempt to describe different fields within the stratum entirely arbitrary. Behind this level the stratum pyramidale can be followed round the splenium into the induseum (Pl. 1, fig. 6). Apart from a further and very marked decrease in size, and the complete disappearance of the granule cells of the dentate gyrus, it does not show any further change in structure until the anterior hippocampal cortex is reached above the septum (Breathnach, 1953).

*Subicular cortex.* This is a transition area between the cornu ammonis and the entorhinal cortical area, or, more posteriorly the general isocortex, without clearly defined morphological features which distinguish it sharply from adjacent areas. It occupies most of the summit of the hippocampal gyrus (Pl. 1, fig. 4). In Pl. 2, fig. 8, its structure is illustrated from a region which, in our judgement, lies about mid-way between well-defined entorhinal cortex and the cornu ammonis, and where five laminae are distinguishable. The first (molecular) contains a considerable number of fine myelinated fibres, which may represent part of the 'perforant path' of Lorente de No (1934). The second, third and fourth are made up of similar cells, pyramidal or fusiform in shape and of moderate size. These cells are more closely packed in the second and fourth lamina than in the third. The fifth lamina is a broad zone of rather small, scattered polymorphous cells.

Traced from the cornu ammonis this kind of cortex seems to be formed by a splitting up of the stratum pyramidale; in the entorhinal area, the lamination pattern becomes slightly more complex and more clearly defined (Pl. 1, fig. 2). We could find no structural criteria by which the subicular area could be divided into a number of different fields such as the pre-subiculum, para-subiculum, etc., of Lorente de No (1934), although more detailed analysis in silver and Golgi preparations might show that they exist. It must also be admitted that the subiculum in the porpoise bears only a superficial resemblance to the various subicular areas illustrated by Rose (1926) and Lorente de No (1934). It might be possible to fit the scheme of lamination adopted by either of these authors to the condition in the porpoise, but this could probably be done in quite a number of cortical areas. The identification of subiculum depends essentially on its topographical position between the cornu ammonis and the entorhinal area. Whether the term should be extended



to include transitional cortex in the posterior part of the hippocampal formation (Pl. 1, fig. 5) or the induseum (Pl. 1, fig. 6), must, in the absence of intrinsic structural criteria, be left an open question.

*Induseum griseum.* This is quite well developed in the porpoise. It is shown in Pl. 1, fig. 6, and no further description is necessary. The size and structure illustrated are preserved with little or no change throughout the whole length of the corpus callosum.

### *Fibre systems*

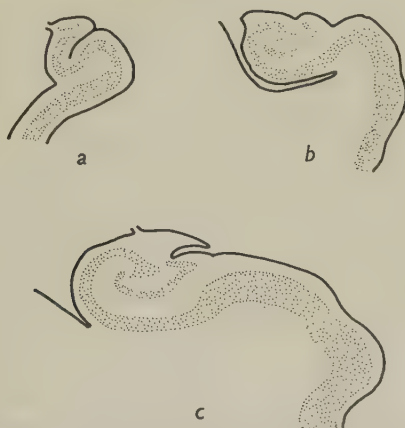
Very little can be added under this head. The alveus layer of fibres is not conspicuously different in thickness from the similar layer in man and in the sheep. The fimbria in Pl. 1, fig. 4, is very small, but this section is taken from near the anterior end of the hippocampus; more posteriorly it is much larger (Pl. 1, fig. 5). This apparent increase in size is partly due to some obliquity in the plane of section, and without counting the number of fibres it is not possible to make reliable comparisons with the size in other mammals. As the fornix, beneath the corpus callosum, it appears to be little smaller than in man, but the fibres which descend behind the anterior commissure to the mammillary region are comparatively few in number. A rather large proportion of fornix fibres take a pre-commissural course in the porpoise (Breathnach, 1953).

### *The dimensions of the hippocampal formation*

Clearly the most satisfactory dimension for comparative purposes would be the total volume of the hippocampal formation. Attempts to estimate this were made using the same planimetric methods as for the amygdala, but were not found satisfactory. The indefiniteness of the boundary, particularly along the subicular margin, was found to introduce quite a large uncertainty into the estimate of the sectional area of the hippocampus; moreover, one could have no confidence that a point selected as the boundary in the porpoise really corresponded with any precision to a point similarly selected in another mammal. In such circumstances it became clear that only large differences of size could be considered significant, and that approximate estimates obtained by simpler means would not be improved by planimetry of a large number of sections.

Another difficulty was caused by the extent of the so-called vestigial part of the hippocampus in the porpoise, i.e. that part in which no differentiated dentate gyrus accompanies the small cornu ammonis and subiculum. Much of this is in the induseum, but a considerable amount is found in the posterior part of the subcallosal hippocampus. In the account which follows we have not attempted any quantitative comparison with other mammals so far as this part of the hippocampal formation is concerned. It appears to form rather a larger proportion of the formation in the porpoise than in most mammals, but, partly because of its shape and lack of differentiation, it is difficult to obtain measurements which could be used for comparison. Attempts at quantitative comparison have therefore been limited to that part of the subcallosal hippocampus in which a definite granular lamina of the dentate gyrus could be recognized in addition to a cornu ammonis and subicular region.

It was found that the antero-posterior extent of the hippocampus in which a dentate gyrus could be recognized either macro- or microscopically, was 12 mm. The corresponding measurement in the sheep is 31 mm. and in the human brain 50 mm. Text-fig. 9 illustrates transverse sections of the hippocampus of the porpoise, sheep and man, all drawn at the same magnification and taken from the region where the cross-sectional area appears largest. It is obvious that this area in the porpoise is much the smallest of the three. Estimates, which must be approximate on account of the uncertainty of the exact position of the subicular border, show that the area in the sheep is about twice and in man about 4 times that in the porpoise. When one



Text-fig. 9. Tracings, made to a uniform scale, of cross-sections through the hippocampal formation in (a) the porpoise, (b) the sheep, and (c) man. The sections were selected in each case from the region of maximal development.

considers also that the length of the fully differentiated hippocampus in the sheep is  $2\frac{1}{2}$  times and in man 4 times that in the porpoise, it becomes obvious that the differences in total volume must be very large indeed. One can indicate the order of these differences by saying that the hippocampus in the sheep must be about 5 times and in man more than 10 times, as voluminous as in the porpoise, and these figures are not likely to be significantly altered by any probable error in the measurements. They become particularly striking when one remembers that the total volume of the sheep brain is about a quarter of that of the porpoise and that human brain is only about 3 times larger. One can conclude that the fully differentiated part of the hippocampus is absolutely smaller and relatively very much smaller than in the sheep or man.

While statements that the hippocampus of the Cetacea is small are common in the literature, the only other measurements we have found are those of Hill (1893). In a brain of *Phocaena* he gives figures only for the lengths of certain parts of the hippocampal formation, e.g. 10 mm. for the 'length of the folded portion of the cortex, the hippocampus proper', which corresponds reasonably well with our estimate of 12 mm. for the length of that part of the hippocampus in which the granular lamina of the dentate gyrus could be recognized. The other dimensions given by Hill do

not differ significantly from measurements which could be made in our specimens, and do not need further discussion.

One may sum up this account of the hippocampal formation in the porpoise as follows. Qualitatively, in Weigert and Nissl preparations, it does not differ significantly from the hippocampus of other mammals; the absence of a hippocampal flexure cannot be regarded as an important point. It might be a result of the unusual shape of the skull and brain in the Cetacea, or possibly of the relatively small size of the hippocampus. The fact that cells in the stratum granulare and stratum pyramidale of the dentate gyrus and cornu ammonis respectively are rather less closely packed than usual, and the strata themselves somewhat less clearly defined, gives an impression of deficient differentiation. Rose's figures (1926), however, show that there is a considerable variation in this respect among mammals in general, and the significance of a somewhat superficial observation of this kind is doubtful. The wider spacing of cell bodies might result from a better development of the dendritic field, and if so, could be evidence of higher differentiation rather than regression.

There remains as its outstanding characteristic the very small size of the hippocampus, both absolute and relative to the size of the brain as a whole. At a very conservative estimate it cannot be more than a half, and probably much less than a half the volume of the hippocampus in mammalian brains of comparable size. In making this statement, however, one must remember that we have very little knowledge of the relative size of the hippocampus in some of the larger mammals with highly convoluted cortices. The relatively large amount of the hippocampus contained in the induseum, etc., where no dentate gyrus is differentiated, is also striking; the total length of the subcallosal hippocampus, for example, is 21 mm., but a dentate gyrus is recognizable even microscopically, for no more than 12 mm. of this distance. It should be added that the fibre system formed by the fimbria and fornix does not seem to be reduced in proportion to the hippocampus as a whole, but more precise methods for estimating its size, or the number of fibres it contains are necessary before any more definite statement can be made.

#### THE PREPYRIFORM AND ENTORHINAL CORTICAL AREAS

Several previous workers (Kukenthal & Ziehen, 1889; Riese, 1924; Langworthy, 1932) are agreed that the organization of the cetacean cortex differs considerably from that of other mammals. It is therefore even more difficult than usual to establish homologies for cortical areas in these animals. It is possible, however, to define the general position (but not the precise boundaries) of the areas under consideration by macroscopic, topographical criteria, and it becomes of interest to determine whether the cortex found in these positions shows a characteristic structure, and if that structure resembles in any way that of similarly placed cortex in other mammals.

*Prepyriform area.* The frontal part of the prepyriform area has already been described (Breathnach, 1953); it can be followed posteriorly into the Sylvian fossa (Text-figs. 1-5), where it becomes increasingly difficult to identify, being represented only by a molecular layer deep to which are a few scattered cells with no definite evidence of lamination. Beyond this point it bends on itself to become the temporal prepyriform area on the upper surface of the temporal operculum



(Text-figs. 3, 4). Throughout, the molecular or zonal layer contains myelinated fibres, and this is in fact the main structural criterion by which this cortex can be defined. If olfactory bulbs were present, such fibres would be identified as belonging to the lateral olfactory tract.

It is clear that the prepyriform cortex of the porpoise is very similar to that of the lateral olfactory gyrus of man, and that it extends to a position corresponding to the limen insulae and then turns forward on the upper surface of the temporal lobe towards the amygdala. While the anterior or frontal portion is moderately well represented, the temporal extension is extremely rudimentary.

*Entorhinal cortex.* In most mammals this cortex occupies the posterior part of the pyriform lobe (the area pyriformis posterior or post-pyriform cortex) where it is separated from isocortex by the posterior part of the rhinal fissure. An intervening area peri-rhinalis is distinguished by some authors (e.g. Rose, 1926). Medially and anteriorly it is separated from the cortical amygdaloid nucleus by the cortico-amygdaloid transition area (periamygdala of Rose, 1926) and medially and posteriorly by the subiculum from the cornu ammonis.

A well differentiated cortical area with these relationships can be seen in the porpoise, and part of its extent is shown in Text-figure 8. To low-power examination it presents a characteristic appearance as two well-defined cellular laminae separated from each other by a relatively cell-free zone (Text-figs. 1-7). More detailed examination shows that six laminae can be distinguished as follows (Pl. 1, fig. 2): (1) molecular, containing an occasional nerve cell; (2) a layer of pyramidal cells which are closely packed and in places arranged in clusters or islands; (3) a broader layer containing similar cells but much more widely spaced; (4) a narrow layer containing comparatively few small polygonal cells; (5) a closely packed layer of large polygonal cells; (6) an ill-defined 'polymorphous' layer with rather widely scattered cells of varying shape.

A similar pattern can be distinguished in all parts of the entorhinal area, although there are obvious regional variations. For example, a section taken from the upper surface of the temporal lobe (Pl. 1, fig. 3) is superficially very different from the section (Pl. 1, fig. 2) taken from the hippocampal gyrus. The same laminae can be recognized, but they are less clearly defined; cells are in general more widely separated and the total depth of the cortex is much greater. To what extent these differences can be attributed to differences in the plane of section it is difficult to say; the greater apparent depth, the broadening of the laminae and their loss of clear definition might well be due to this. The spacing of the cells would probably not be greatly affected by the plane of section, but no useful purpose would be served by attempting to define subdivisions in the entorhinal area without the use of more critical histological methods.

The six laminae described can be made to correspond reasonably well with those described by Lorento de No (1933). It is also true to say that the description in four laminae adopted by Rose (1926) can be fitted to the condition in the porpoise; but it is clear that the actual number of laminae distinguished in almost any part of the mammalian cortex is to some extent arbitrary, especially when only Nissl material is available. Our findings demonstrate that, in the porpoise, there is a well differentiated and extensive cortical area which occupies the same topographical position

as the entorhinal cortex of other mammals. Its intrinsic structure, so far as this can be studied in Nissl material, is similar to that of the entorhinal cortex, and the differences in detail which exist are probably no greater than could be found between any two mammals of widely different species. In other words, the evidence for the identification of this area as entorhinal is as good as in many other mammals, and it may be pointed out in addition that its extent and high degree of differentiation in the porpoise is in striking contrast to the rudimentary condition of the prepyriform cortex.

#### THE RETROSPLLENIAL AND CINGULATE CORTICAL AREAS

The remaining cortical areas and certain parts of the diencephalon will be described very briefly. A more detailed account could be justified only as part of a wider survey of the cortex or thalamus as a whole; here only features relevant to their possible relationship to the rhinencephalon need be considered.

*Retrospllenial area.* In the retrospllenial region a small area of cortex having the structure illustrated in Pl. 2, fig. 9, can be identified. It lies along the subicular border of the induseum, and could with some justification be referred to as a 'granular' cortex. In general its cellular elements are smaller than in most regions of the isocortex of the porpoise (compare Pl. 2, fig. 11, cingulate cortex, and Pl. 2, fig. 12, from the isocortex of the frontal lobe), and a considerable number are of the 'granule' type. A somewhat similar cortex extends in a subspllenial position along the hippocampal gyrus towards the entorhinal area (Pl. 2, fig. 10). Here there is also a predominance of small elements so that one is reminded of the 'koniocortex' of von Economo (1929).

The retrospllenial cortex is obviously more distinctly laminated than the cortex in a subspllenial position, but it is very doubtful if an attempt to define these laminae and compare them in detail with those of other mammals could be justified. It is enough for our present purpose to point out that a cortex in which small or 'granular' elements are prominent is characteristic of this situation in many other mammals, including man.

*Cingulate area.* The cingulate gyrus is well developed, and the histology of its cortex is illustrated in Pl. 2, fig. 11. It is fairly clearly laminated, and there is a marked absence of granule cells. The lamination is rather similar to that of the frontal cortex (Pl. 2, fig. 12) and each could be fitted to either of the two slightly different schemes of lamination proposed by Riese (1924) and Langworthy (1932). The cingulate cortex is uniform throughout the length of the gyrus; there is no differentiation of granular and agranular regions as in other mammals.

#### DIENCEPHALIC STRUCTURES

*The hypothalamus.* Only the mamillary region need be considered here, though it may be said in passing that the hypothalamic nuclei in general conform to the usual mammalian pattern. The mamillary bodies are represented by slight accumulations of small cells on each side of the midline close to the floor of the third ventricle. A medial nucleus can be recognized, but more on account of the position of the cells medial to the fibres of the fornix than because they form a distinct group. No other cell groups could be recognized, so that in this respect the

mamillary region is relatively undifferentiated. It has already been noted that the fornix fibres which reach the mamillary region are few in number, and it can be added that in our material no clearly defined mamillo-thalamic tract can be recognized. Hatschek & Schlesinger (1902) also had difficulty in identifying this tract in *Delphinus delphis* though Langworthy (1932) was able to follow it to the anterior thalamic nuclei in *Tursiops truncatus*.

*The thalamus.* Only the anterior group of nuclei have been examined in detail, and these are illustrated in Pl. 2, fig. 7. The identifications must be regarded as tentative, since the groups of cells found in the anterior tubercle of the thalamus are very irregular and quite unlike the well-defined cell groups found, for example, in the sheep. They extend in the series only through about 0.7 mm. in a cranio-caudal direction, after which they are replaced by a large dorso-medial nucleus. It must be admitted that the posterior boundary of the cell group marked antero-ventral nucleus is very difficult to define; it may extend a little further posteriorly than appears to be the case, but even if it does the very small size and poor differentiation of the anterior group of nuclei as a whole remains a conspicuous feature of the porpoise thalamus. The absence of a defined mamillo-thalamic tract has already been noted.

*Epithalamus.* The remarkable development of the habenular ganglia and commissure and the fasciculus retroflexus has been noted by previous authors (Hatschek & Schlesinger, 1902). Medial and lateral nuclei are readily distinguishable; the cells of the former stain deeply and are closely packed; those of the latter are pale and are scattered on a dense plexus of fibres. Apart from its greater size, the epithalamus of the porpoise is essentially similar to that of other mammals.

#### DISCUSSION

The present paper completes the description of the 'rhinencephalon' of an anosmatic brain, and two main questions arise to which the findings should be relevant, namely: (i) What changes in structure can be attributed to the loss of secondary olfactory connexions? (ii) What inferences concerning the neurological aspect of olfactory function in mammals generally can be drawn from an examination of a brain in which such functions are absent?

In the rabbit and monkey Clarke & Meyer (1947) and Meyer & Allison (1949) have shown that fibres from the olfactory bulb end in a part of the anterior olfactory nucleus and olfactory tubercle, the prepyriform cortex, the nucleus of the lateral olfactory tract, and in the cortical medial and central amygdaloid nuclei. In addition a few such fibres end throughout the bed nucleus of the stria terminalis, some after crossing in the anterior commissure. It is probable that this description applies generally to osmatic mammals.

If the differences which can be observed in these structures in the porpoise can all be attributed to the loss of secondary olfactory connexions, it is at once clear that this loss is very variable in its effects. These range from almost complete regression to vestiges which cannot be recognized with confidence, as in the anterior olfactory nucleus and the nucleus of the lateral olfactory tract, to an apparent absence of any substantial change as in the cortico-medial amygdaloid nuclei or the bed nucleus of the stria terminalis. The olfactory tubercle formation and the



prepyriform cortex occupy an intermediate position, showing marked retrogression in extent but enough characteristic differentiation to be clearly recognizable.

To some extent such variations may be related to variations in the concentration of olfactory tract connexions. For example, Meyer & Allison (1949) have shown that in the monkey the temporal prepyriform cortex receives more olfactory tract fibres than the frontal, and it is the temporal part of this area in the porpoise which shows the most marked retrogression. It is obvious, however, that, to varying degrees, all the structures so far considered must play a part in olfaction in osmatic mammals, although their structural diversity shows that there must be corresponding functional differences. Some may be concerned only with the relay and reinforcement of impulses of olfactory origin, and these one would expect to disappear or become vestigial as a result of the loss of olfactory receptors. This appears to have been the fate of the olfactory bulb, the anterior olfactory nucleus and the nucleus of the lateral olfactory tract. Cortical areas or complex nuclear groups may be concerned with the discrimination and integration of impulses from many different sources, and, through efferent connexions, may have acquired control of mechanisms which are not activated solely by one type of stimulus. In this connexion it is relevant to point out that Kaada (1951), in monkeys, cats and dogs, found that 'marked inhibition of respiratory movements was produced on stimulation of points in the amygdaloid nuclei', as well as from a number of other regions most of which are included in the 'rhinencephalon' of the older literature. Inhibition of respiration in response to certain olfactory stimuli may clearly be important; in an aquatic mammal respiratory inhibition is equally or more important as a preparation for submergence, and if the amygdala is a neural mechanism capable of this function, it is not surprising that it should undergo no retrogression in spite of a lack of olfactory connexions.

One may suggest, therefore, that the anterior olfactory nucleus and the nucleus of the lateral olfactory tract should be classed with the olfactory bulb as structures concerned exclusively with the analysis, relay or reinforcement of impulses of olfactory origin, but that the amygdala, including its cortico-medial nuclei, is not primarily olfactory in function, though it may be activated by olfactory stimuli in osmatic mammals. The significance of the prepyriform cortex of the porpoise is more difficult to assess. Its great reduction in this animal, and the abundance of its connexions from the lateral olfactory tract in osmatic mammals, leave no doubt as to its primary functional association with olfaction. There is no evidence on which to base even speculations concerning the function of the remnants of this cortex in the porpoise.

It is doubtful if any of the remaining parts of the rhinencephalon receive direct connexions from the olfactory bulb in any mammal. They could not be demonstrated in the rabbit (Clark & Meyer, 1947) or the monkey (Meyer & Allison, 1949). There is no reason to think that these animals are atypical in this respect, and it is now generally accepted that statements, common in the older literature, that secondary olfactory fibres reach the septum, hippocampus and entorhinal cortex, were based on inadequate evidence.

It is, however, obvious that the absence of direct connexions from the olfactory bulb does not exclude the possibility of olfactory function. The entorhinal cortex,

for example, is very closely associated with the prepyriform cortical area, and might well receive tertiary olfactory connexions from that source. Such fibres, however, have been shown to be few in number (Allison, 1953), and comparative studies suggest that the entorhinal cortex reaches its greatest extent and highest degree of differentiation in the microsmatic Primates (Rose, 1927; Allison, 1953). That the entorhinal area does receive some impulses of olfactory origin in macrosmatic mammals is therefore likely, but it is clear that its structural differentiation is not dependent on these connexions, a conclusion which receives confirmation from the observation that it is extensive and well differentiated in the anosmatic porpoise. It may be that the entorhinal cortex is in some respects similar to the amygdala, capable of activation by olfactory impulses when these are present, but performing functions which lose none of their importance when they are absent. If so, the fact that the entorhinal cortex is well developed in the porpoise, in spite of the absence of an olfactory bulb is not surprising. In other mammals, however, the entorhinal cortex is thought to give origin to the majority of the afferent fibres to the hippocampus. It is worth noting, therefore, that a small and apparently retrogressive hippocampus need not be associated with a corresponding reduction in the entorhinal cortex.

One of the most striking features of the porpoise brain is the remarkably small size of the hippocampal formation. Some authors (e.g. Addison, 1915) have associated this with the loss of the sense of smell, as was reasonable when the hippocampus was thought to serve predominantly olfactory functions. Now, since the older evidence for olfactory function in the hippocampus has been found to be inadequate (Brodal, 1947*a*), the mere presence of a recognizable hippocampus in an anosmatic mammal is taken as additional proof that it is not concerned with olfaction in any mammal. While one cannot avoid being impressed by the coincidence that a structure which has for many years been regarded as olfactory in function, should be so poorly developed in an anosmatic mammal, it is nevertheless clear that the condition in the porpoise can be used to support either hypothesis, olfactory or non-olfactory, for hippocampal function. However, since the amygdala, which undoubtedly receives olfactory connexions in most mammals, is not substantially reduced in the porpoise, it is clearly reasonable to look elsewhere for the cause of the reduction which has occurred in the hippocampus.

This reduction is the more striking since it is accompanied by such marked lack of differentiation in the mamillary region and in the anterior thalamic nuclei, regions which owed their inclusion in the rhinencephalon to their connexion, direct or indirect, with the hippocampus. The cingulate cortex does not show a corresponding reduction, though the absence of any 'granular' area is suggestive. It is difficult to avoid the conclusion that we have here a complex neural mechanism the inter-connected parts of which serve a common function which has become much less important in aquatic mammals. What this function may be is at present almost entirely a matter of speculation. It has been suggested that it is related to the control of emotional reactions (Papez, 1937; Bard & Mountcastle, 1948, and others) and the fact that the hypothalamus is included in the complex suggests a relationship to autonomic function. It is perhaps relevant to point out that an aquatic environment is very uniform so far as conditions of temperature and humidity are concerned,

and this may make a number of autonomic adjustments, vital for a terrestrial mammal, unnecessary. A detailed study of the whole hypothalamus might throw some light on this question.

The large size of the habenular nuclei has been noted; it is therefore unlikely that these are exclusively olfactory in function in any mammal, but more than that it is not possible to say. In fact, the main general conclusion which can be drawn from this whole study is that investigations of the comparative anatomy of the brain, especially when they are based on the rather superficial evidence of Nissl and Weigert preparations, are very rarely capable of precise or reliable functional interpretation. They may provide hypotheses or suggestions which indicate fruitful lines for investigation, in which it will be necessary to use quantitative, experimental or physiological methods. They may provide useful corroborative evidence, although, as in the case of the porpoise hippocampus, this will often be equivocal, and capable of supporting more than one hypothesis. Their main purpose must remain to outline the structural background and to provide ideas and suggestions which may be tested and modified by the more precise methods indicated above.

#### SUMMARY

The amygdaloid nuclei, hippocampal formation, and entorhinal cortical area of the anosmatic porpoise (*Phocaena phocaena*) has been described on the basis of Nissl and Weigert stained serial sections. A less detailed description is given of the cingulate and retrosplenial cortical areas, the mamillary region of the hypothalamus, the anterior thalamic nuclei, and the habenular ganglia.

The amygdaloid nuclei resemble very closely those of other mammals, except for the probable absence of the nucleus of the lateral olfactory tract. Estimates of volume show that the cortico-medial group forms approximately the same proportion of the whole complex as in the sheep.

The hippocampal formation, while showing all the parts characteristic of this formation in mammals in general, is very small. Approximate estimates indicate that the part of the formation in which both a cornu ammonis and a dentate gyrus can be recognized has about one-fifth of the volume of the corresponding structures in the sheep, and one-tenth those in man. The vestigial parts of the hippocampus (e.g. induseum griseum), in which no differentiated dentate gyrus can be recognized, appear to form a larger proportion of the whole formation than in other mammals, and a considerable part of the subcallosal hippocampus is in this condition. There is no hippocampal flexure; the fornix is well developed, but comparatively few of its fibres reach the mamillary region.

The entorhinal cortex shows no signs of regression; it is characteristically differentiated, and extensive.

Of the remaining structures examined, only the mamillary region and anterior thalamic nuclei show conspicuous differences from other mammals; in both, nuclear differentiation is very poor as compared, for example, with the sheep, and a mamillo-thalamic tract is not recognizable as a defined bundle of fibres.

Taking into account the observation previously reported (Breathnach, 1953) it is concluded that the only structures whose loss or regression can be related to the loss of olfactory function are the olfactory bulb, the anterior olfactory nucleus, the



prepyriform cortex, the nucleus of the lateral olfactory tract, and possibly the cortex of the olfactory tubercle. The cortico-medial amygdaloid nuclei show no significant change, and probably do not owe their primary functional significance to olfactory connexions, although these are present in osmotic mammals. Regression in the hippocampal formation and in the related mamillary and anterior thalamic nuclei is a characteristic feature of the porpoise brain, but there is no evidence to suggest that this is the result of the loss of olfactory function.

The material for this study was provided through the kindness and co-operation of the Director of the Marine Station, Millport, Isle of Cumbrae. Our thanks are due also to Mr Rex Jarrett who prepared the sections and the microphotographs which illustrate this paper, and to Prof. J. Jansen who allowed us to see the typescript of his paper on the amygdaloid complex of the fin-whale before publication.

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#### EXPLANATION OF PLATES

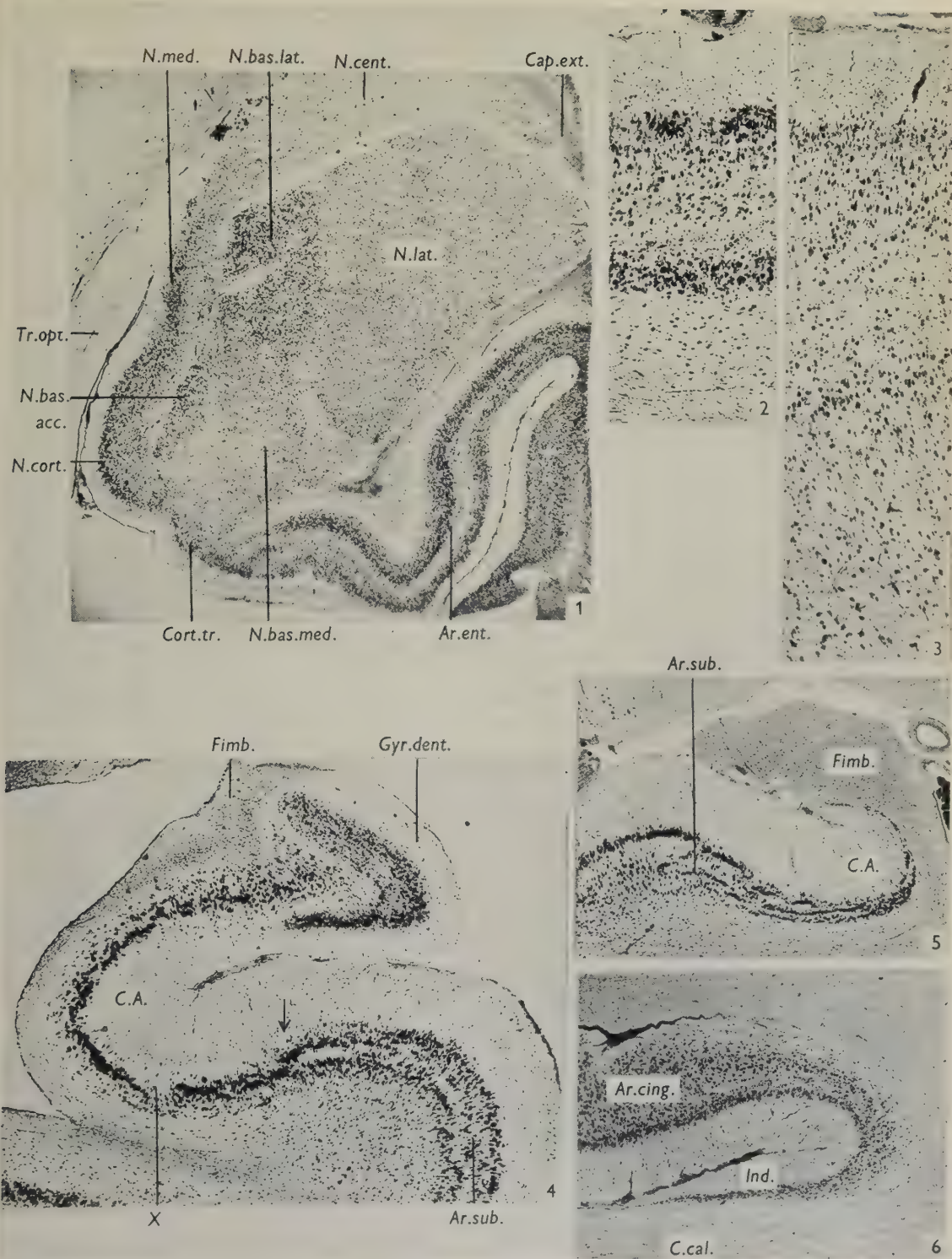
All photographs are of celloidin sections 20  $\mu$  thick, stained with thionin.

##### PLATE 1

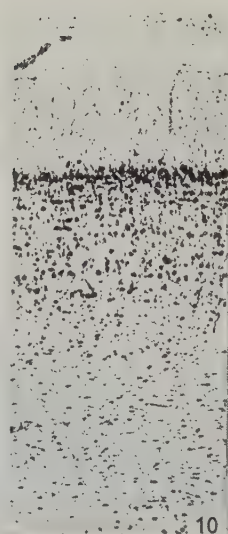
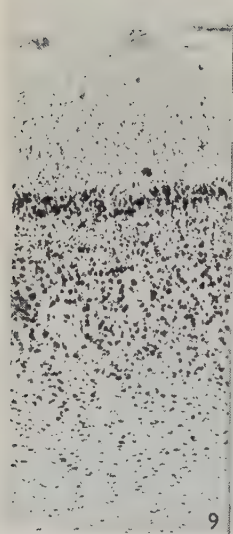
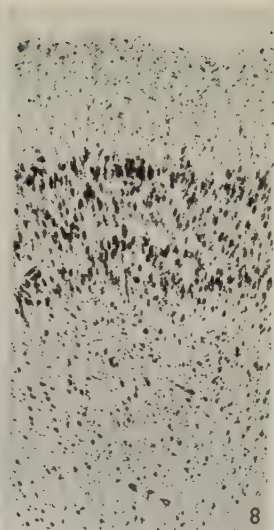
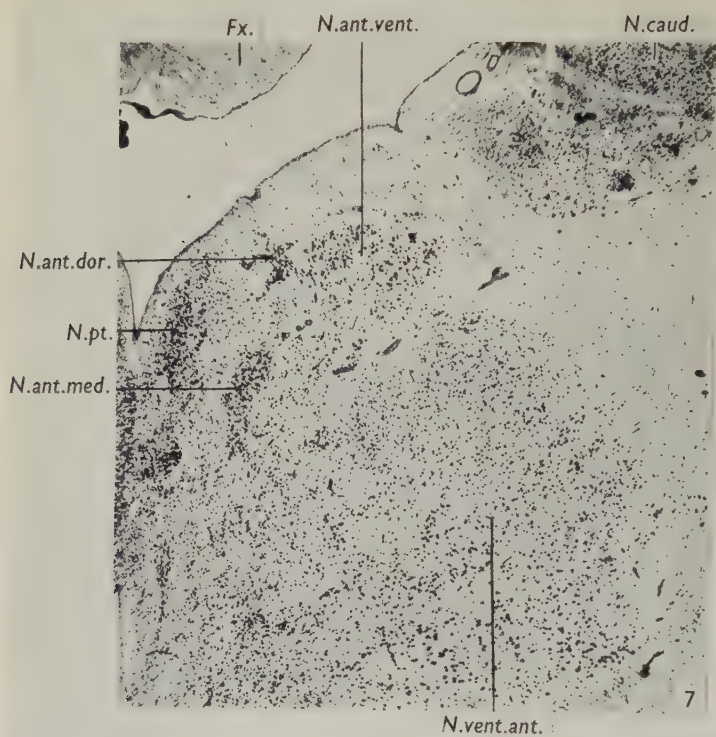
- Fig. 1. An oblique section through the amygdala in which all the nuclei are represented.  $\times 8$ .
- Fig. 2. The cortex of the entorhinal area on the hippocampal gyrus.  $\times 32$ .
- Fig. 3. The cortex of the entorhinal area on the upper surface of the temporal lobe.  $\times 32$ .
- Fig. 4. The hippocampal formation cut at the region of maximal development. X marks the break in the cell layer referred to in text (p. 278). The arrow indicates the probable point of transition between cornu ammonis and subicular cortex.  $\times 23$ .
- Fig. 5. A section through the posterior part of the hippocampal formation about 10 mm. anterior to the splenium.  $\times 16$ .
- Fig. 6. The induseum griseum.  $\times 16$ .

##### PLATE 2

- Fig. 7. A section through the anterior part of the thalamus.  $\times 12$ .
- Fig. 8. The subicular cortex.  $\times 32$ .
- Fig. 9. The cortex of the retrosplenial area.  $\times 32$ .
- Fig. 10. The cortex of the hippocampal gyrus posterior to the entorhinal area.  $\times 32$ .
- Fig. 11. The cortex of the cingulate gyrus.  $\times 32$ .
- Fig. 12. A section of the cortex on the dorso-lateral aspect of the anterior part of the frontal lobe, close to the mid-dorsal line.  $\times 32$ .







LIST OF ABBREVIATIONS  
USED IN TEXT-FIGURES AND PLATES

<i>Ar.ant.</i>	Anterior amygdaloid area	<i>N.bas.med.</i>	Medial part of basal amygdaloid nucleus
<i>Ar.cing.</i>	Cingulate area		
<i>Ar.ent.</i>	Entorhinal area	<i>N.cent.</i>	Central amygdaloid nucleus
<i>Ar.ins.agr.</i>	Agranular insular area	<i>N.cort.</i>	Cortical amygdaloid nucleus
<i>Ar.prep.</i>	Prepyriform area	<i>N.lat.</i>	Lateral amygdaloid nucleus
<i>Ar.sub.</i>	Subicular area	<i>N.med.</i>	Medial amygdaloid nucleus
<i>C.A.</i>	Cornu Ammonis	<i>N.ant.dors.</i>	Antero-dorsal thalamic nucleus
<i>C.cal.</i>	Corpus callosum	<i>N.ant.med.</i>	Antero-medial thalamic nucleus
<i>Cap.ext.</i>	External capsule	<i>N.ant.vent.</i>	Antero-ventral thalamic nucleus
<i>Cort.tr.</i>	Cortico-amygdaloid transition area	<i>N.caud.</i>	Caudate nucleus
<i>F.am.</i>	Amygdaloid fissure	<i>N.lent.</i>	Lentiform nucleus
<i>Fimb.</i>	Fimbria	<i>N.pt.</i>	Parataenial nucleus
<i>Fx.</i>	Fornix	<i>N.tr.ol.</i>	Nucleus of lateral olfactory tract
<i>Gyr.dent.</i>	Dentate gyrus	<i>N.vent.ant.</i>	Anterior ventral thalamic nucleus
<i>Ind.</i>	Induseum griseum	<i>Str.ter.</i>	Stria terminalis
<i>Mass.int.</i>	An intercalated cell mass	<i>Tr.opt.</i>	Optic tract
<i>N.bas.acc.</i>	Accessory basal amygdaloid nucleus		
<i>N.bas.lat.</i>	Lateral part of basal amygdaloid nucleus		

# THE OPTIC TECTUM OF *GALLUS DOMESTICUS*: A CORRELATION OF THE ELECTRICAL RESPONSES WITH THE HISTOLOGICAL STRUCTURE

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## INTRODUCTION

The optic tectum of birds is a suitable preparation for studying the electrical responses of both neuronal dendrites and perikarya. The action potential of the perikarya of spinal motor neurones has been described in detail by Brock, Coombs & Eccles (1952), but the flow of current between perikarya and their processes in a complex tissue remains uncertain. The extent of the surface area of dendrites compared with the neuronal perikaryon has recently been investigated by Sholl (1953) in a quantitative study of mammalian cerebral cortex. He has estimated that the dendritic surface area constitutes 85–95 % of the total surface area of the whole neurone.

In the optic tectum of birds, several zones may be seen, each characterized by a particular dendritic orientation. In some zones, the dendrites run predominantly parallel to the surface, and in others they are mainly perpendicular. We have taken advantage of these features to attempt an analysis of the electrical responses of both the perikarya and their dendrites. The method has been to record the electrical response to stimulation of the optic nerve at different depths in the tectum. Although other afferent connexions to the tectum besides the optic nerve have been described by P. Ramón y Cajal (1943) and others, we have not utilized them in the present study.

Several previous attempts have been made to analyse the electrical responses at different depths in central nervous structures, usually by the monopolar method of recording. Thus Lorente de Nó (1947) found changes with depth in the electrical responses of the hypoglossal nucleus, and Burns & Grafstein (1952) claim to have localized in depth the neurones responding to local stimulation of mammalian cerebral cortex. The optic tectum of the duck and the goose was investigated by O'Leary & Bishop (1943), and in this work both monopolar and radial pairs of electrodes were used. Some change of the electrical response with depth was reported but the exact relationship to the histological structure was not established.

## METHODS

Adult chickens of various breeds and both sexes were used in this work. The staining methods employed were Nissl, Golgi-Cox and Bielschowsky-Gros silver, all the material being removed from the living animal under pentobarbitone (Nembutal) anaesthesia. For the Nissl preparations the tectum was fixed in Sansom's Carnoy solution (absolute alcohol, 65 ml.; glacial acetic acid, 5 ml.; chloroform 30 ml. corrosive sublimate to saturation) double-embedded in celloidin-



paraffin and sections cut at  $10\mu$  thickness. These were stained with polychrome-methylene blue (Borrel's blue), which in our experience proved more satisfactory than thionin. The modification of the Golgi-Cox method used was that described by Sholl (1953): specimens were fixed for 7 weeks, embedded in celloidin and cut at  $120\mu$  thickness. Silver preparations were obtained from formol-fixed specimens, the Bielschowsky-Gros method used being a modification after Richardson (personal communication). The sections were cut in frozen material at thicknesses varying from 10 to  $20\mu$ .

#### *Quantitative methods*

The densities of nerve fibres and perikarya were estimated at successive depths from the tectal surface. In order to express cell or fibre density in terms of unit volume, the area surveyed was multiplied by the section thickness. To facilitate comparison all values were then standardized at a volume of  $10^6\mu^3$ . Measurements of section thickness were made throughout the tectal depth, four random samples being taken at each level.

For counts of perikarya in Nissl preparations a continuous strip of tectum  $100\mu$  wide was surveyed from the pial surface to the ependyma of the ventricle. The principal difficulty encountered was the differentiation between neuroglial nuclei and those of small neurones. In the case of intermediate and large neurones, where the cytoplasm was visible, the presence of Nissl bodies was taken as the criterion. Where differentiation depended on nuclear characteristics alone the presence of a definite nucleolus in a vesicular nucleus, or infolding of the nuclear membrane was considered to indicate that the cell in question was a neurone. The neuroglial nuclei showed no nucleolus but rather scattered chromatin substances. Infolding of the nuclear membrane was absent and these nuclei tended to be more darkly stained than those of the neurones (Spielmayer, 1922, p. 139). Endothelial and fibroblast nuclei stained more darkly still.

In the Golgi-Cox preparations a survey of the total nerve fibre density from the pial surface to a depth of  $1400\mu$  was carried out. This was done in continuous strips 1 mm. wide. A binocular microscope was used with cross-wires in one eyepiece in addition to the graticule in the other. Fibres were counted by means of the graticule and the cross-wires were used to classify them as radial or tangential, according to whether they were more nearly perpendicular or parallel to the tectal surface. The few fibres orientated about  $45^\circ$  to the surface were ignored.

Fibre counts and classification into tangential and radial groups were carried out in the same way in silver preparations. Owing to the fact that by this staining method nearly all fibres are shown it was not practicable to use continuous strips throughout the depth of the tectum. Samples were taken of fibre density in  $20,000\mu^3$  volumes at  $50\mu$  intervals throughout the tectal thickness.

#### *Experimental procedures*

##### *Surgical approach to the tectum and optic nerve*

After premedication with atropine (0.6 mg.) the animals were anaesthetized with pentobarbitone sodium (Nembutal) injected into a vein on the ventral surface of the wing. In chickens the dose of pentobarbitone sodium necessary for surgical

anaesthesia is close to the lethal dose. Repeated small doses had to be given in order to maintain the required level of anaesthesia over the period of almost 2 hr. necessary for the experiment. The dose required varied considerably and was controlled by observations on the respiratory rate. The head was fixed by two clamps, one applied to the beak and the other to the external auditory meatus. A mid-line dorsal incision was made and the skin reflected laterally. The periosteum in the region extending from the posterior orbital margin to the occiput was stripped with a diathermy needle: this procedure greatly reduced subsequent bleeding from the bone. The tectum was exposed by nibbling away the spongy bone in the region immediately dorsal to the external auditory meatus. Great care was taken on reaching the dura to avoid pressure on the underlying tectum and reflexion of the former was delayed until immediately before insertion of the electrodes.

As a preliminary to exposure of the contralateral optic nerve the eyeball was eviscerated and the periosteum stripped from around the orbit. The dorsal and posterior orbital margins were removed with rongeurs, then slight traction on the sclera enabled the nerve to be exposed. Again the opening of the dural sheath was delayed until the commencement of electrical recordings. At all stages bleeding was controlled by the use of muscle grafts and bone wax.



Text-fig. 1. Photomicrographs of examples of radial (left) and tangential (right) microelectrodes.  $\times 30$ .

### *Recording of electrical responses*

(a) *Recording electrodes.* Since the unit producing electrical responses is the neurone with a cell body of some  $10\text{--}20\mu$  in diameter, it is desirable to use recording electrodes of similar dimensions in order to relate the electrical responses to the histological structures producing them. Microelectrodes were therefore made with shaft diameters of  $10\text{--}20\mu$  by reducing steel needles electrolytically by the method of Grundfest, Sengstaken, Oettinger & Gurry (1950). Four coats of insulating varnish were baked on these needles, and the tips were then cut off with scissors under a dissecting microscope. Such electrodes were held in a micromanipulator which enabled them to be advanced or retracted axially in steps of  $10\mu$ . An example of the track made by such electrodes is shown in a Golgi-Cox preparation (Pl. 4, fig. 13).

Monopolar recordings from such electrodes cannot be expected to exhibit the electrical results of the radial and tangential fibre orientations which are such a marked feature of the structure of the tectum. Voltage gradients have therefore been measured in two directions at right angles by recording the voltage difference between pairs of these microelectrodes set some  $200\mu$  apart, either radially or tangentially (Text-fig. 1). This separation of the electrodes was chosen when it was found that electrodes closer together than this gave inconveniently small voltage differences. A separation of  $200\mu$  is of the same order as the distance between the perikaryon of a tectal neurone and the more distant ramifications of its dendrites.

(b) *Stimulating electrodes.* The optic nerve was stimulated in the orbit by a needle electrode thrust into the optic nerve. Silver tubes concentric with the needle were connected to the anode of the stimulator circuit and to the earth, and these made contact with the tissues of the orbit. The shaft of the needle and the sides of the tubes were insulated with varnish except at the ends which were platinized. The needle electrode was connected to the cathode of the stimulator circuit, and pulses of rectangular wave-form and some 0.2 msec. duration were used. These were delivered through a radio-frequency isolating transformer similar to that described by Schmitt & Dubbert (1949). Pulses of 10–50 V. peak amplitude were used, though the response was usually maximal at about 25 V.

(c) *Recording.* The amplifiers were of the type described by Bishop & Harris (1950). They were used with a time-constant of about 1 sec., and had a discrimination ratio against in-phase signals of 1000 to 1. The signal was applied to the grids of a balanced pair of cathode followers (Bishop, 1949). The trace was photographed directly at full size on recording paper.

## RESULTS

Previous workers have investigated the histology of the bird's optic tectum from a purely structural standpoint. Division into laminae has been based principally on cell types seen in Golgi-Cox preparations and to some extent on fibre arrangements. The layers described vary from six (Van Gehuchten, 1892) to fifteen (P. Ramón y Cajal, 1943), and other workers have identified intermediate numbers (Bellonci, seven (1888), Von Kölliker, nine (1896) and Ris, nine (1899)). The classification into fifteen layers proposed by Ramón y Cajal seems to us to be excessive in that it does not throw much light on the functional organization of the tectum as a whole. Furthermore, in our Golgi preparations no such elaborate subdivision appears possible and we have used van Gehuchten's interpretation as a basis for our work.

Our histological investigations and electrical recordings have been made from that part of the tectum extending from the pial surface to the ventricle. A general view of this region, stained by the silver method, is shown in Pl. 1, fig. 1. The total thickness varies from about 1.8 to 2 mm. and this variation is mainly accounted for by diminution of the optic nerve layer as its fibres spread out over the tectal surface and also to a certain extent to differences in the depth of the central fibre layer. The intermediate cellular layers maintain a fairly constant depth.

### *The optic nerve layer*      *Histological structure*

This is found just below the pial surface and contains fibres from the contralateral retina which, after crossing in the chiasma, spread over the tectum. After leaving the chiasma, the fibres reach the cranio-ventral extremity of the tectum and as they radiate pass caudally, laterally and dorsally. At the region where it first reaches the tectum this layer is about  $400\mu$  thick, but diminishes rapidly to about  $100\mu$  as its fibres diverge. Whereas in the silver preparations (Pl. 1, fig. 1) the optic nerve layer appears as a zone of closely packed fibres, in specimens stained by the Nissl method only the nuclei of the neuroglial cells are shown (Pl. 1, fig. 2).

In Golgi preparations the arrangement of the optic nerve fibres and their terminal



aborizations have been described by Van Gehuchten (1892) and P. Ramon y Cajal (1943). In our material, stained by the Golgi-Cox method, we were able to confirm the presence of these ramifications, which, however, are not found at levels deeper than the superficial plexiform layer.

*The superficial plexiform layer.* This is about  $130\mu$  thick and contains as well as the optic nerve terminations, small neurones of the stellate type. These are best seen in the Golgi-Cox preparations which show their cell processes to be predominantly orientated in the tangential plane (Pl. 2, fig. 3). In some cases, however, a single fine process, probably the axon, passes centrally into the radial fibre layer. Here it forms part of the pattern of radially orientated fibres characteristic of the zone. In silver preparations the combination of the stellate cell processes with the ramifications of the optic nerve terminations produces an appearance in which no specific fibre orientation can be seen (Pl. 2, fig. 4).

*The radial fibre layer.* This had a depth of  $600-700\mu$  and shows most strikingly in Golgi-Cox preparations where three main cell types may be identified in it. The commonest of these has a small fusiform perikaryon and two prominent dendrites, passing superficially and deeply in the radial plane (Pl. 2, fig. 5). The second cell type, also frequently seen, has a large pyramidal perikaryon. These are found principally in a zone about the middle of the radial layer. The apical dendrites are usually two to three in number and arise either directly from the cell body or by the division of a single trunk close to the perikaryon. To the left of Pl. 3, fig. 6, a pyramidal type neurone with the proximal parts only of the apical dendrites is shown, while in the case of the neurones in Pl. 4, fig. 11, the full extent of these dendrites may be seen. They extend into the superficial plexiform layer where they break up into several branches. These apical dendrites contribute to the predominantly radial arrangement of fibres in this region. The basal dendrites of the pyramidal cells are variable in number and pass in all directions (Pl. 3, fig. 6; Pl. 4, fig. 11). The third cell type, fewest in number, is of the small stellate variety as seen to the right in Pl. 3, fig. 6. The processes of these are relatively short, run in all directions and branch freely. In addition to the dendritic processes of the three types of neurones which have been described, finer branches are also seen arising from the perikaryon or from the main dendrite near the cell body. Only occasionally, however, could one of these be identified positively as the axon.

In Bielschowsky-Gros silver preparations the predominantly radial orientation of the fibres in this layer is confirmed (Pl. 1, fig. 1). At higher magnifications, however, additional features, not demonstrated by the Golgi-Cox method are seen. In the more superficial region of the radial layer some fine fibres are present, many of these being orientated away from the radial plane (Pl. 3, fig. 7). The calibre of these is much smaller than the radial fibres already identified as dendrites in Golgi-Cox preparations. In the deeper part of the radial layer fibre density increases and under high power numerous discrete fibre bundles are identified both in the tangential and radial planes (Pl. 3, fig. 9). In view of the relatively few axons stained in our Golgi-Cox preparations we consider that the large number of additional fine fibres seen in the silver material must be axons. Certainly in the deeper part of the radial layer many of the fibres are grouped in bundles, which is an arrangement typical of incoming or outgoing axons. Furthermore, the individual fine fibres mentioned above can

frequently be traced to one of these axon bundles. In the silver preparations thick tangential fibres are seen at a level corresponding to the position of the pyramidal cell bodies described in the Golgi-Cox material (Pl. 3, fig. 8). These probably are the basal dendrites of these cells (although the perikarya themselves are not stained).

On examining Nissl material it was found that the radial layer showed a high cell density compared with the other layers (Pl. 1, fig. 2). These cells are distributed in three laminae within the radial layer, the two superficial ones being discrete rows of a few cells in depth, whereas the third and deepest layer occupies a zone of about  $250\mu$  thickness throughout which the cells are evenly distributed. It is in this deep layer that the majority of the pyramidal cell bodies are to be found: the fusiform and stellate cells are distributed throughout all three laminae.

#### *The deep plexiform layer*

This is  $400\text{--}500\mu$  in depth and again the fibre pattern is well demonstrated in Golgi-Cox preparations (Pl. 2, fig. 5; Pl. 4, fig. 10). The neurones are all of the large stellate variety with long dendrites, many of these being disposed about the tangential plane. Some of the dendrites pass into the deeper parts of the radial layer, but most of them intermingle in their own layer providing its characteristic plexiform appearance. We have been unable to demonstrate the axons of these cells in Golgi-Cox preparations, although they have been described by previous workers who traced them into the central fibre layer (Van Gehuchten, 1892; Ramón y Cajal, 1911, p. 212).

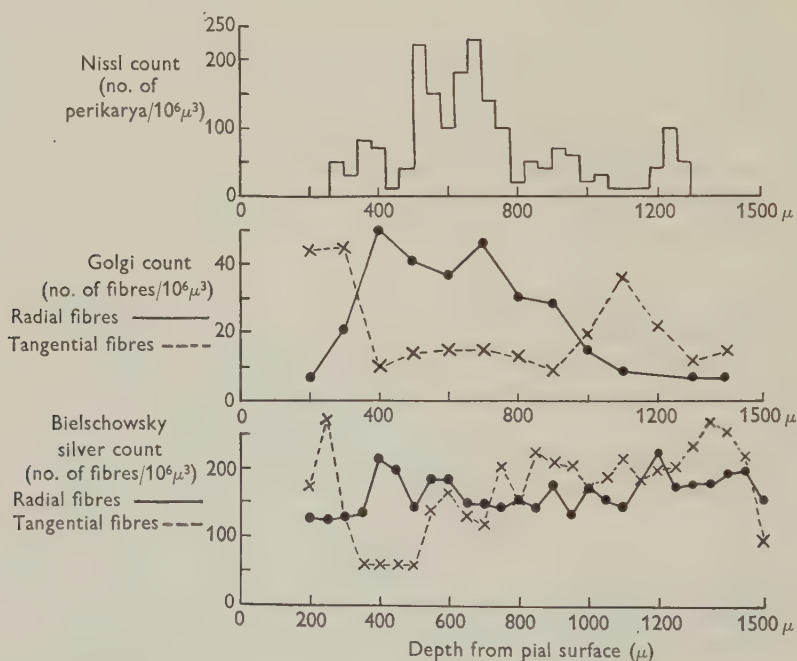
In silver material the prominent feature of this layer is the presence of numerous large bundles of axons, some of which are radially and others tangentially directed. The frequent crossing at right angles of these bundles gives this region a very characteristic appearance (Pl. 3, fig. 9). In the almost rectangular intervals thus formed the large nerve cell bodies may often be seen. Also in Nissl preparations this region gives a characteristic picture. The perikarya are large and well spaced out: they are polygonal or oval in shape and their cytoplasm contains abundant coarse Nissl bodies.

*The central fibre layer.* This varies from  $250$  to  $450\mu$  in thickness and contains both incoming and outgoing axons. The structure of this layer has been described by Ramón y Cajal (1911, p. 208), who considered that the majority of the fibres were efferent but that there were some afferent axons having their terminations in the cellular layers of the tectum. The destination of the efferent axons has not yet been adequately investigated. In our work we have not been concerned with the connexions of these fibres, but rather with their orientation. In silver stained material we have traced the radial axon bundles of the deep plexiform layer into this zone where, at varying levels they turn sharply to run ventrally or dorsally in the tangential plane. In parasagittal sections of the tectum they are therefore cut transversely, and it can be seen that they have a thick myelin sheath (Pl. 4, fig. 12).

*The periventricular layer.* This is  $50\text{--}80\mu$  in thickness and contains scattered neurones of the stellate type. This zone corresponds to the periventricular grey layer of mammals (Kappers, Huber & Crosby, 1936) which, however, in birds is greatly reduced. It is limited internally by the ependyma of the ventricle.

*Quantitative analysis of cell and fibre distribution**Distribution of perikarya in Nissl preparations*

A histogram showing the numbers of perikarya in volumes of  $10^6 \mu^3$  through the entire depth of the tectum is shown in Text-fig. 2 (Nissl histogram). Six such surveys were made, and this example is typical of the series. Slight deviations from this distribution occur, and these may be attributed to small differences in tectal structure and to variations in the relative depth of the individual layer. The first peak at  $300\text{--}400 \mu$  and the last peak at  $1200\text{--}1300 \mu$  correspond to the superficial and deep plexiform layers respectively. In the intervening region the several subpeaks cannot be attributed to specific cell laminae in the radial layer owing to the variation in thickness of the tectum and its individual cell layers already mentioned. However, the high cell density of the radial layer is indicated on the histogram in the  $500\text{--}800 \mu$  region.



Text-fig. 2. The distribution according to depth from the pial surface of the number of perikarya (Nissl preparation) and of fibres (Golgi-Cox & Bielschowsky-Gros preparations) are shown in separate groups.

*Distribution and orientation of dendrites in Golgi-Cox preparations*

Although representing density per unit volume graphs have been plotted in preference to histograms owing to the difficulty of reading the latter when two have to be superimposed. It will be seen in Text-fig. 2 that in these preparations the ratio of radial to tangential fibres varies considerably throughout the depth of the tectum. The radial fibres show a single broad peak from  $400$  to  $700 \mu$ , whereas in the case of the tangential fibres two peaks are seen, one at  $200\text{--}300 \mu$  and the other at



1000–1200  $\mu$ . The large radial peak corresponds to the radial fibre layer, the two tangential peaks reflect the superficial and deep plexiform layers respectively.

#### *Distribution and orientation of fibres in silver preparations*

Here again the graphic representation has been preferred to histograms for the reason already mentioned. It is noticeable in Text-fig. 2 that the ratio of radial to tangential fibres is much more constant throughout the depth of the tectum than in the case of the Golgi-Cox preparation although near the surface a small tangential followed by a radial peak is seen. We attribute the much more uniform ratio between the two classes of fibres in silver preparations to the presence of axons which mask the great contrast in orientation of dendrites seen in the different layers of the Golgi-Cox preparation.

As a result of the qualitative and quantitative findings, the neural layers of the tectum may be conveniently divided into three main zones. These are the superficial plexiform layer, the radial fibre layer and the deep plexiform layer, the classification being based on the orientation of dendrites. The sharp differences between these layers make the tectum a suitable centre in which to attempt to correlate electrical responses with histological structure.

### ELECTRICAL RESPONSES

When the voltage difference between a microelectrode in the tectum and an indifferent electrode on the skull was recorded, little spontaneous activity was seen, the peak amplitude being usually less than 100  $\mu$ V. The contralateral optic nerve was stimulated electrically in the orbit, and a single shock was found to evoke a brief spike and two longer waves of potential change in the tectum. The first spike occurred 0.65 msec. after the stimulus, and was of positive polarity, but very small amplitude, often less than 100  $\mu$ V. The first of the longer waves began 1 msec. after the stimulus, reached a peak 1.5 msec. later, and thereafter declined to zero in 2.5 msec. When the microelectrode was at the surface of the tectum, the polarity of this wave was positive, the peak amplitude about 600  $\mu$ V. The second of the longer waves was of the opposite polarity to the first, being negative at the surface. It began immediately after the first wave had declined to zero amplitude, that is about 5 msec. after the stimulus. The duration of this second wave varied from 10 to 30 msec. and the peak amplitude of 400  $\mu$ V. was reached some 4 msec. after the trace crossed the base-line. The voltage amplitudes mentioned were the largest that could be evoked, and shocks of threshold strength elicited smaller tectal responses, without, however, any apparent change in the polarity or timing of the waves. Text-fig. 3 shows this pattern of response recorded at different depths in the tectum with a focal microelectrode, and maximum stimulus amplitude. There is very little change in the amplitudes, polarities, or latencies of the two response waves at different depths in the tectum.

#### *Radial voltage gradients*

When the voltage difference between a radial pair of microelectrodes was recorded at different depths in the tectum after stimulating the optic nerve, a response of similar time-course to the monopolar recording was found (Text-fig. 4). The

amplitude of the gradient rose to a peak at about  $500\mu$  below the pia, and declined above and below this maximum, without change of polarity, in a manner which was approximately exponential. These radial electrodes were connected so that the lower one deflected the trace upwards in the photograph when it went negative to the upper one. The voltage gradients found show that the deeper electrode was positive to the upper electrode during the first wave of the response. Only very

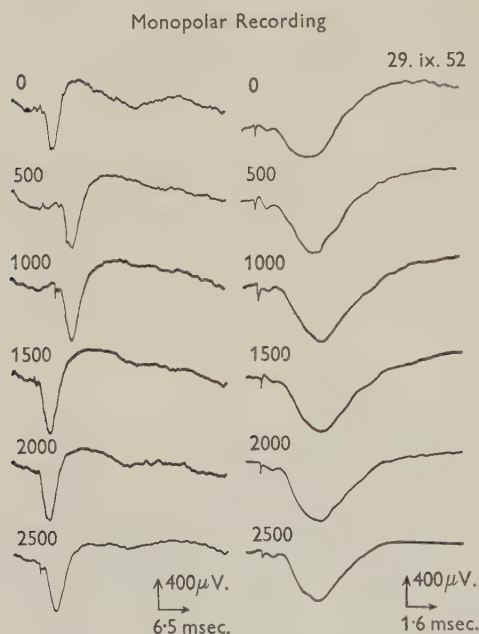


Fig. 3.

Text-fig. 3. Recordings made with a single focal electrode at  $500\mu$  intervals throughout the depth of the tectum. The two columns show the same responses on a slow (left) and fast (right) time-base.

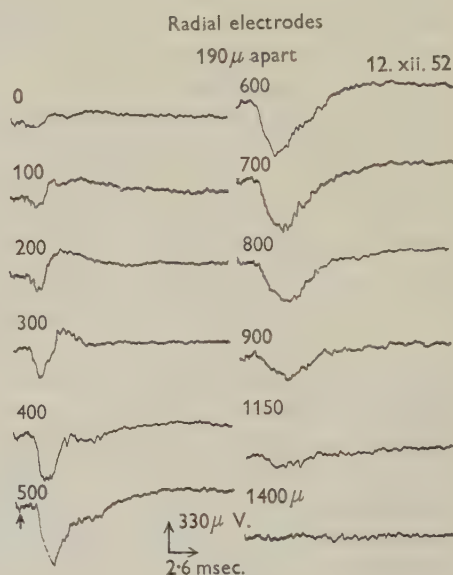
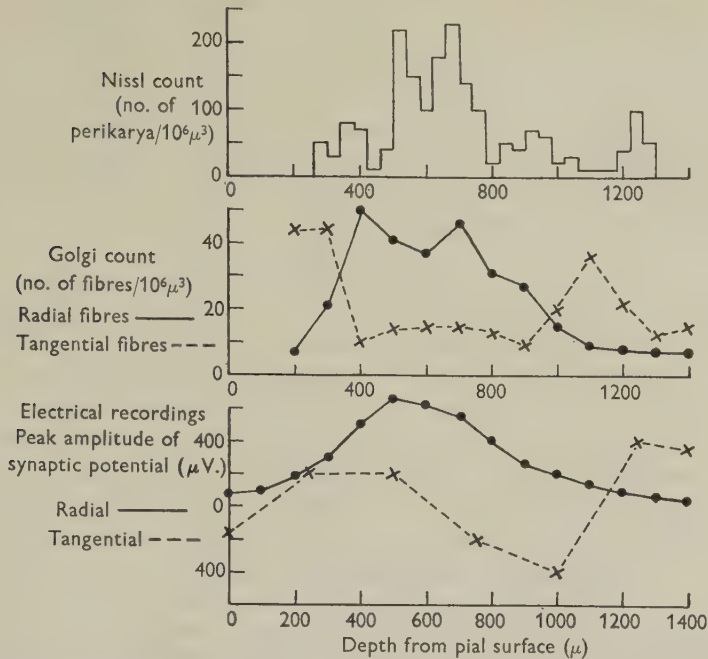


Fig. 4.

Text-fig. 4. Recordings made with two focal electrodes having a radial separation of  $190\mu$ . Most of the records were taken at  $100\mu$  intervals.

small voltage gradients were found at deeper levels in the tectum among the large stellate cells. The peak amplitude of this first wave of response was measured on the photographs, and in Text-fig. 5 this voltage gradient has been plotted against the distance below the pia. It is seen to correspond with the number of radial dendrites counted in the Golgi-Cox preparation, and plotted in the same figure. The peaks of these two distributions coincide at about  $500\mu$  below the pia, where the cell-bodies of the radial neurones, counted in the Nissl preparation and plotted as the top line in the figure, are most numerous. This distribution of the radial voltage gradient has, on the other hand, little apparent connexion with the distribution of axons and dendrites counted together in the silver stained preparation (Text-fig. 2) and it appears, therefore, that the radial voltage gradient is not related to the distribution of axons, but only to the dendrites and cell-bodies.



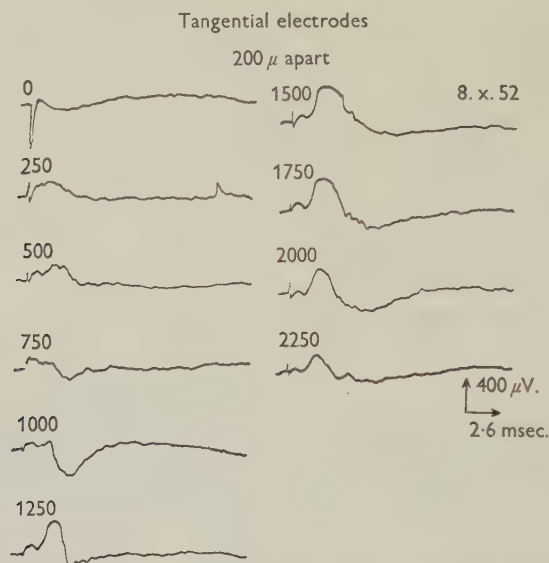
Text-fig. 5. The upper two graphs show the perikaryon (Nissl preparation) and fibre (Golgi-Cox preparation) distribution as in Text-fig. 2. In the lowest graph the peak amplitude of the first wave of the response recorded with the radial and tangential electrodes is plotted against the same horizontal depth scale.

### Tangential voltage gradients

Recordings have also been made from tangential pairs of microelectrodes for which there is a choice in the direction of alinement. We have found that electrodes alined in the direction in which the optic afferent fibres run over the tectal surface record large tangential voltage gradients, whereas electrodes alined at right angles to this direction record very little change of voltage. Text-fig. 6 shows the records obtained from a tangential pair of electrodes alined in the direction of the optic nerve fibres, and connected so that the one nearer the chiasma deflected the trace upwards in the photograph when it went negative to the one further from the chiasma. It is seen that a reversal of small voltage gradients occurs between 0 and 250  $\mu$  below the pia, and a reversal of larger gradients between 1000 and 1250  $\mu$  below the pia. In each case these voltage gradients are superficially positive to negative, and more deeply, after the reversal, negative to positive in the direction of the optic afferent fibres. It is seen also that the tangential voltage gradients are very small in the region from 500 to 750  $\mu$  below the pia in which the radial voltage gradients are maximal. The peak amplitude of these tangential voltage gradients during the first wave of response has been measured from the photographs and plotted against the depth below the pia in Text-fig. 5. This distribution is to be compared with the number of tangential dendrites counted in the Golgi-Cox preparation and with the number of cell bodies counted in the Nissl preparation, plotted in the



same figure. It is seen that in both plexiform layers, the peak count of tangential dendrites is superficial to the peak count of cell bodies of tangential neurones, and the positive to negative voltage gradients occur above the negative to positive gradients. There is, however, little similarity between the distribution of tangential voltage gradients and the counts of axons and dendrites together in the silver preparation, plotted in Text-fig. 2 except possibly at the surface. It seems, therefore, that it is the cell bodies and dendrites rather than the axons which are to be related to the electrical responses.



Text-fig. 6. Recordings made with two focal electrodes having a tangential separation of  $200\mu$ . Records were taken at  $250\mu$  intervals.

## DISCUSSION

An account of the electrical responses of the avian optic tectum has been published by O'Leary & Bishop (1943). These authors, working on the duck and the goose, used microelectrodes in which some  $200\text{--}500\mu$  of the shaft was exposed. This large exposure was perhaps responsible for the unsatisfactory correlation obtained between the electrical recordings and the histological structure. An interpretation of our electrical recordings will now be given in terms of the histological structure of the tectum and the action potentials of neurones.

### *Interpretation of the electrical responses*

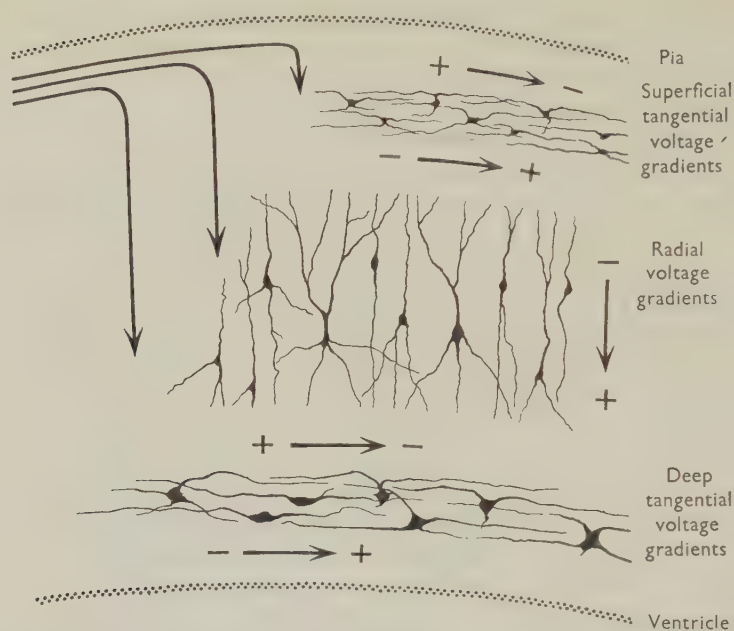
The brief spike seen  $0.65$  msec. after the stimulus to the optic nerve in the orbit persisted for a few minutes after the death of the animal, when the later waves of response were abolished. It is interpreted as the arrival of synchronized impulses in the optic nerve fibres at the tectum, and with a conduction path of  $2\text{--}3$  cm. from the orbit, this corresponds to a velocity of  $30\text{--}45$  metres/sec. For comparison, Hursh (1939) reported velocities of this order in pre-ganglionic cervical sympathetic nerves of the cat containing fibres of  $6\text{--}8\mu$  maximum outside diameter. Adrian & Matthews

(1927) report that the largest fibres in the optic nerve of the conger eel are of  $8\mu$  outside diameter, and Bishop (1953) has found a similar result in the optic nerve of the cat. The interpretation offered is therefore consistent with what is known of the histology of the optic nerve.

The later response waves are due to tectal neurones, since they involve large voltage gradients within the tectum. A description of the action-potentials of perikarya has been given by Brock, *et al.* (1952), who recorded from single motoneurones in the cat spinal cord. These authors found that the outside of the perikaryon went negative to its more distant surroundings during the synaptic potential, and positive during the later after-potential. The synaptic potential had a time-constant for decay of 3 msec., and the after-potential had a duration of some 100 msec. If the tectum contained neurones with these time-constants in synaptic connexion with the optic afferent fibres, a synchronized volley of impulses in the optic nerve would evoke synaptic and after-potentials in many tectal neurones, which, being integrated together and recorded from the tectal surface, would generate two waves of potential change similar in time-course and polarity to those actually recorded. The interpretation of the two waves of response found is then that they represent the integrated synaptic and after-potentials of the tectal neurones first stimulated by impulses in the optic afferent fibres.

The radial voltage gradients were recorded from a layer of neurones whose dendrites extend both upwards and downwards but mainly in a radial direction. The superficial processes of these neurones nearest to the optic afferent fibres would be depolarized by impulses in the latter before the parts of the neurones lying further down in the radial layer. A negative to positive downward voltage gradient would then be set up during the first wave of the response, and this would have the same time-course and polarity as the gradient which we have found experimentally. Our interpretation is that the radial voltage gradient found during the final wave of the response is due to the depolarization of radial neurones spreading downwards from the surface afferent fibres, as illustrated by the diagram shown in Text-fig. 7.

The tangential voltage gradients were positive to negative among the dendrites superficial to the cell bodies, and negative to positive below among the cell bodies and dendrites, in the direction of the optic afferent fibres. Brock, *et al.* (1952) found that the outside of spinal motoneurones becomes negative to distant indifferent tissue during the synaptic potential. The dendrites receiving part of the current flowing from the cell body during the synaptic potential in the latter will then become positive to their surroundings. Neurones which have been stimulated will then have positive dendrites and negative cell bodies during the first wave of the response. The neurones nearer the chiasma will be stimulated by afferent impulses before the more distant neurones so their dendrites will become positive to the dendrites of unstimulated neurones, and their cell bodies negative as illustrated in Text-fig. 7. There would then be superficial positive to negative tangential voltage gradients, and deeper negative to positive gradients similar to those which we have described above. Our interpretation is that stimulation proceeds through the layer of tangential neurones in the direction of the optic fibres, as well as downwards in the radial layer, and that the dendrites of stimulated neurones become positive and the cell bodies negative, during the first wave of the response.



Text-fig. 7. Diagram illustrating the spread of impulses through the three main layers of tectal neurones according to the hypothesis set out in the text.

#### SUMMARY

1. A correlation has been described between the histological structure of the optic tectum of the chicken and the character of the electrical responses at different depths.

2. The responses were elicited by electrical stimulation of the contralateral optic nerve and recordings in the tectum made with pairs of focal microelectrodes arranged to measure radial or tangential voltage gradients.

3. Histological examination shows a superficial layer of incoming optic nerve axons and a deep zone composed mainly of efferent fibres. The intervening cellular region shows three main laminae exhibiting markedly differing orientation of dendritic processes. There is a superficial plexiform layer showing a predominantly tangential dendritic arrangement, then a zone in which most of the fibres are radial in direction and finally a deep plexiform layer where again a tangential orientation of dendrites is found.

4. The voltage gradients recorded with tangentially orientated electrodes showed peaks at depths corresponding to the position of the superficial and deep plexiform layers. Radially orientated electrodes gave a single peak at the level of the radial fibre layer. An interpretation has been suggested correlating the histological and electrical findings.

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photographic and histological work and for this we are most grateful. Our thanks are also due to the Nuffield Foundation for their generosity which has made this work possible.

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# EXPLANATION OF PLATES

Abbreviations: *c.f.l.*, central fibre layer; *d.p.l.*, deep plexiform layer; *f.c.*, fusiform cell; *o.n.l.*, optic nerve layer; *p.c.*, pyramidal cell; *p.l.*, periventricular layer; *r.f.l.*, radial fibre layer; *s.c.*, stellate cell; *s.p.l.*, superficial plexiform layer; *v.*, ventricle.

## PLATE 1

- Fig. 1. Bielschowsky-Gros preparation of a coronal section of the optic tectum extending from the pial surface to the ventricle.  $\times 80$ .
- Fig. 2. Nissl preparation of a coronal section of the optic tectum extending from the pial surface to the ventricle.  $\times 80$ .

## PLATE 2

- Fig. 3. Golgi-Cox preparation of the superficial plexiform layer. Some radial dendrites are seen entering this zone in the lower half of the section.  $\times 440$ .

Fig. 4. Bielschowsky-Gros preparation of the superficial plexiform layer. Part of the optic nerve layer is seen in the upper part of the section.  $\times 440$ .

Fig. 5. Golgi-Cox preparation of a coronal section of the optic tectum showing the three principal cell layers.  $\times 84$ .

PLATE 3

Fig. 6. Golgi-Cox preparation of part of the radial fibre layer showing pyramidal, stellate and fusiform cells.  $\times 265$ .

Fig. 7. Bielschowsky-Gros preparation of the radial fibre layer showing fine axons ramifying among the radially directed dendrites.  $\times 380$ .

Fig. 8. Bielschowsky-Gros preparation of the radial fibre layer at the level of the pyramidal perikarya. Although the latter are not visible some of their transversely running basal dendrites are stained.  $\times 380$ .

Fig. 9. Bielschowsky-Gros preparation of the transition zone between the radial fibre and deep plexiform layers. Vertical and transverse axon bundles may be seen.  $\times 550$ .

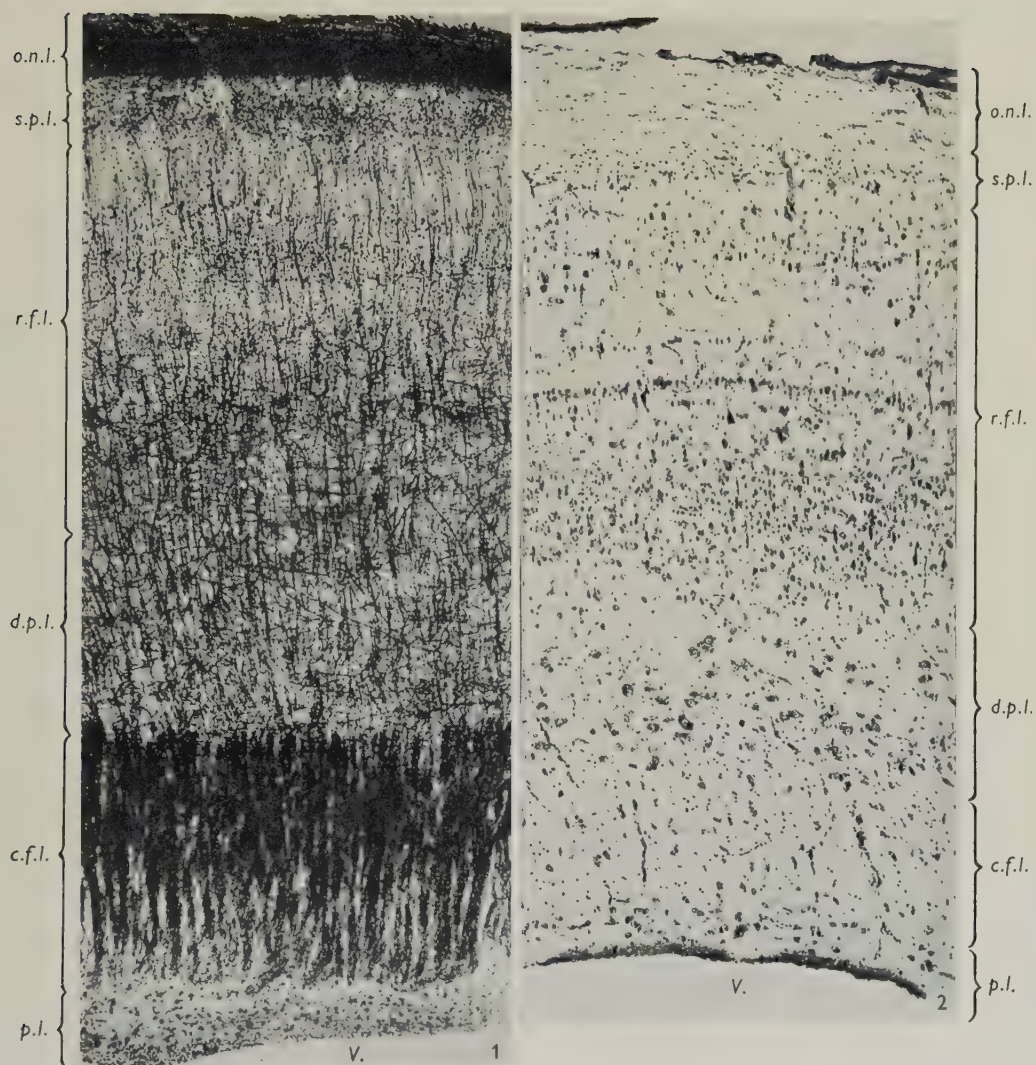
PLATE 4

Fig. 10. Golgi-Cox preparation of the deep plexiform layer.  $\times 104$ .

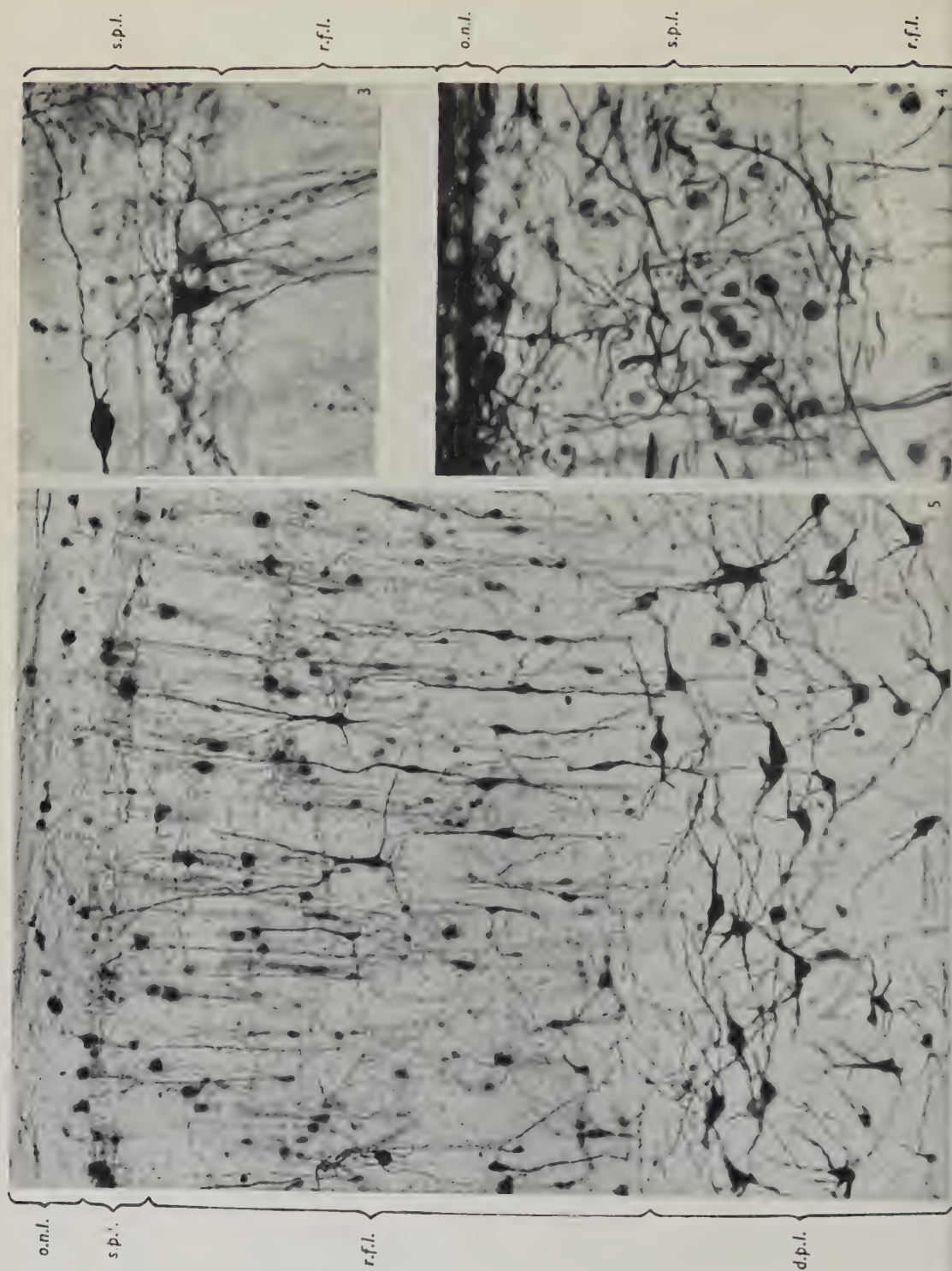
Fig. 11. Golgi-Cox preparation showing the radial fibre and superficial plexiform layers. The basal dendrites of the pyramidal type of cell are well shown.  $\times 212$ .

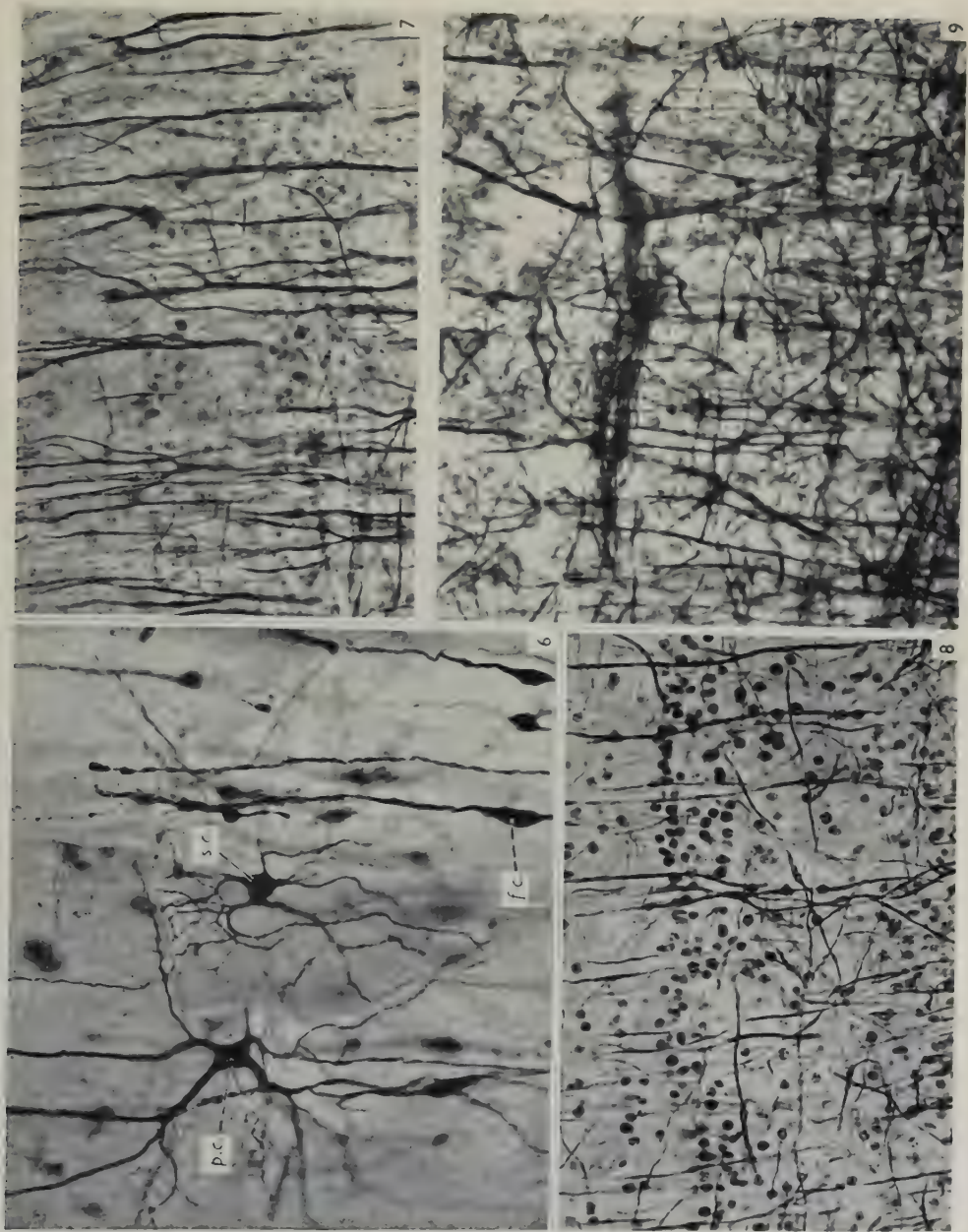
Fig. 12. Bielschowsky-Gros preparation of a parasagittal section of the central fibre layer.  $\times 440$ .

Fig. 13. Golgi-Cox preparation showing part of an electrode track at the junction of the radial and deep plexiform layers.  $\times 84$ .

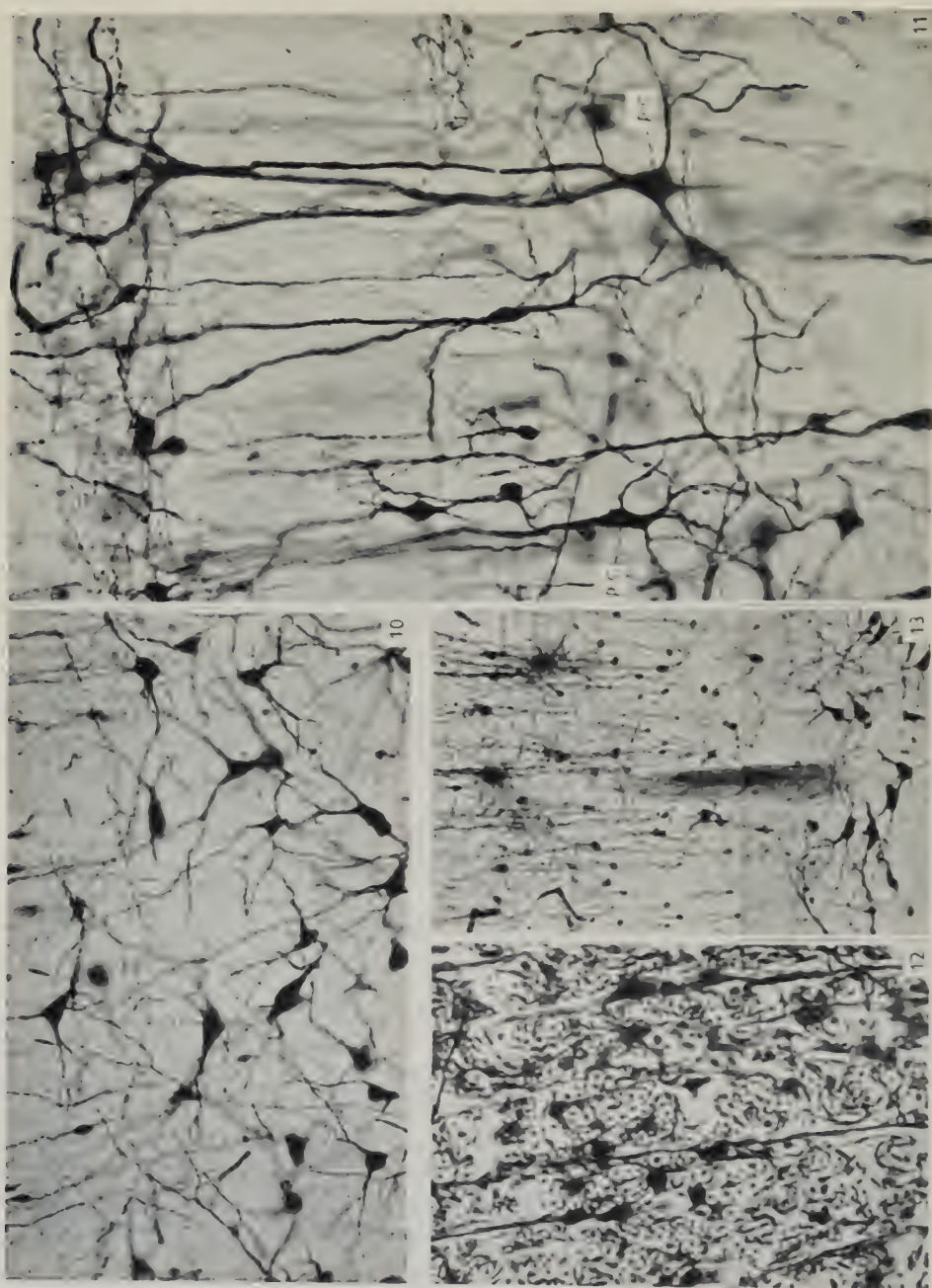














# THE CONNEXIONS OF THE MIDLINE AND INTRALAMINAR NUCLEI OF THE THALAMUS OF THE RAT

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## INTRODUCTION

In 1942, Morison & Dempsey showed that stimulation of the midline and intralaminar nuclei of the thalamus evokes widespread recruiting responses in the cerebral cortex. This has been confirmed and elaborated by Jasper (1949), and the similarity of these responses to those characteristic of *petit mal* epilepsy has been pointed out by Jasper & Droogleever-Fortuyn (1947). These findings are of interest in view of the fact that these nuclei do not degenerate after removal of almost the entire neocortex (Walker, 1938*a, b*; Combs, 1949; Powell, 1952). Rose & Woolsey (1943, 1949), however, have shown that these nuclei will undergo degeneration if, in addition to the neocortex, the so-called 'rhinencephalon' is involved. Other authors (Le Gros Clark & Boggon, 1933; Waller, 1934; Bard & Rioch, 1937; Walker, 1936; Stoffels, 1939*a, b*; Lashley, 1941) have, on occasion, also reported degeneration in some of these nuclei following telencephalic lesions. The only systematic study of the projection of these nuclei is that of Droogleever-Fortuyn (1950), who showed that certain of the midline nuclei are connected with the cortex on the medial surface of the hemisphere, and in a subsequent communication (1951) suggested that the intralaminar nuclei probably project to the striatum. Although Starzl & Magoun (1951) were unable to record recruiting responses from the septum, olfactory tubercle, piriform lobe, amygdala and hippocampus, the head of the caudate nucleus exhibited large recruiting potentials upon stimulation of these thalamic nuclei. Their experimental observations on the thalamic and cortical distribution of these responses led them, however, to suggest that the midline and intralaminar nuclei, together with the nucleus ventralis anterior, function as an intrathalamic association system; a similar conclusion was reached by McLardy (1951) in a review of the anatomical evidence then available. Hanbury & Jasper (1953) have recently reaffirmed that the midline and intralaminar nuclei have a projection independent of the main nuclei and have indicated that the fibres pass forwards from these nuclei through the nucleus ventralis anterior.

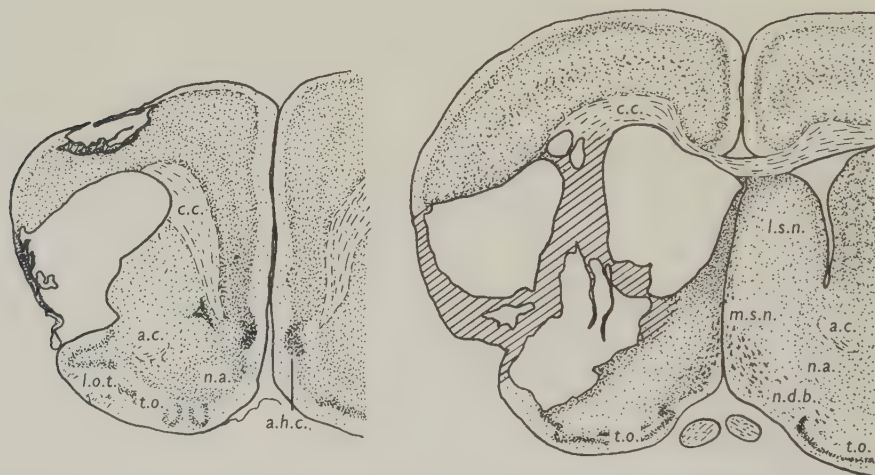
In view of this conflicting evidence it is a matter of some importance that the precise nature of the projection of these nuclei be established. The aims of the experiments to be described here were (*a*) to confirm that the midline and intralaminar nuclei, as well as the main thalamic nuclei, will undergo retrograde degeneration following telencephalic lesions; (*b*) to determine whether these nuclei will degenerate independently of the main nuclei, and (*c*) to define, if possible, the precise projection of these elements. This communication is based on the study of retrograde cell degeneration in the thalamus of the rat following lesions of varying size in the rostral part of the hemisphere.

## MATERIAL AND METHODS

Thirty-one albino rats of various ages were used. The lesions were produced by inserting either a glass-insulated electrode or a fine ophthalmic knife through a small trephine hole over the frontal lobe or through the posterior wall of the orbit after enucleation of the eye. The animals were allowed to survive for periods ranging from 21 days to 7 months. The brains were removed, fixed in 70 % alcohol and 2 % acetic acid and the entire cerebral hemispheres embedded in paraffin wax and cut serially at  $25\mu$ . Most of the brains were sectioned coronally and a few horizontally; every fourth section was mounted and stained with Borrel's methylene blue and every fifth with activated protargol.

## EXPERIMENTAL RESULTS

In many of the experiments the lesions and the resulting degeneration were similar; to facilitate the description such experiments have been grouped together and, while only individual examples of each group will be described in detail, important differences within the groups will be referred to briefly.



Text-fig. 1. Site and extent of lesion in Exp. R6. In this and the following diagrams of lesions, complete destruction is indicated by a clear area and severely damaged regions by hatching.

*Group I.* As most of the available anatomical evidence indicates that the midline and intralaminar nuclei project to the rostral telencephalon, in the first group of three experiments large lesions involving a considerable part of the cortex as well as the basal forebrain areas were placed in the rostral part of the hemisphere. Exp. R6 is an example of this group: the lesion (Text-fig. 1) commences anteriorly at the level of fusion of the pars dorsalis of the anterior olfactory nucleus with the neocortex of the frontal lobe. At this level most of the neocortex on the dorso-lateral aspect of the hemisphere, together with the adjoining part of the pyriform cortex, has been destroyed. More caudally the extent of the damage to the neocortex progressively diminishes to end finally just before the level of the amygdaloid nuclei

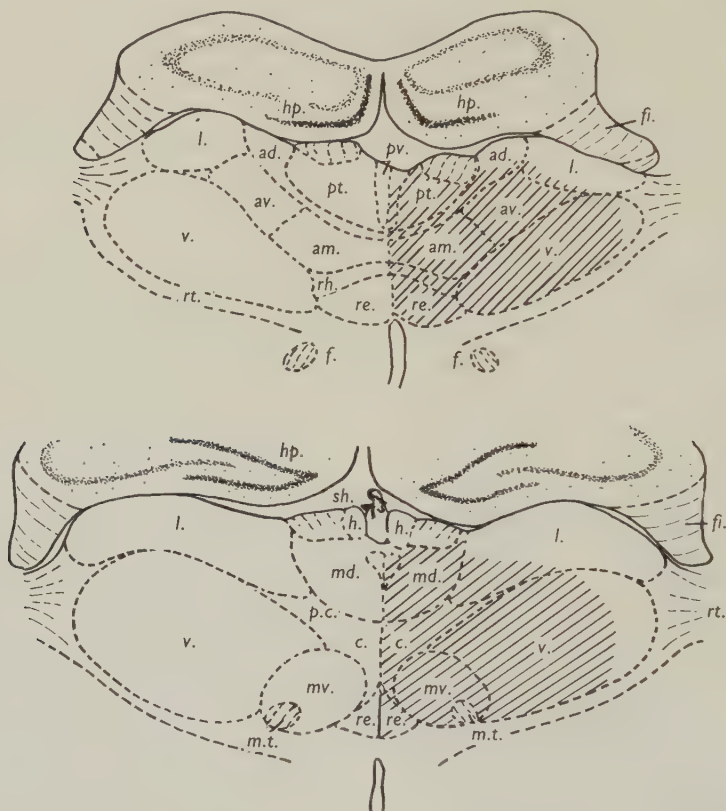
on the normal side. The caudate nucleus, putamen and pallidum have been completely destroyed, except for the most rostral part of the head of the caudate nucleus the fibre connexions of which are however, completely interrupted. The lateral third or half of the nucleus accumbens has been damaged and the anterior limb of the anterior commissure destroyed. Anteriorly the olfactory tubercle has not been involved except for the lateral margin, but at the level of the medial septal nucleus it is completely undercut by the medial extension of the lesion. The anterior third of the septum, together with the anterior hippocampal cortex, is not directly involved; the cells of the lateral septal nucleus are compacted together, however, subsequent to the loss of fibres terminating in and passing through this area. These fibres have been destroyed by the lesion which at the junction of the anterior and middle thirds of the lateral septal nucleus encroaches from the ventricle into the dorso-lateral corner of the septum; the posterior third of the septum is completely destroyed. At no point does the lesion encroach on the septum of the opposite side nor on the hippocampal commissure. The entire medial and lateral preoptic areas, including the bed nucleus of the stria terminalis and the nucleus of the diagonal band, have been ablated; the lesion extends back for a short distance into the anterior hypothalamic area, ending just rostral to the caudal end of the supra-chiasmatic nucleus. In their anterior halves, all the amygdaloid nuclei are completely destroyed. Beginning with the medial and cortical nuclei they reappear in successively caudal sections and in the posterior third of their extent all the nuclei are intact. In front of the thalamus the internal capsule is almost completely destroyed, but opposite the thalamus only the peripheral margin is directly involved.

The thalamus shows severe retrograde cell degeneration (Text-fig. 2). The only nuclei which show little or no change are the antero-dorsal, the lateral half of the antero-ventral, the dorso-lateral part of the ventral, the lateral, the posterior, the pretectal and both parts of the lateral geniculate nucleus. The medial and lateral habenular nuclei show no change either in this or any other experiment. With the exception of the dorsal third of the posterior paraventricular nucleus, all the other dorsal thalamic elements (including the midline and intralaminar nuclei) show very severe degeneration in the form of almost complete cell loss with shrinkage and pallor of the few remaining cells. It should be noted that the cellular changes in that part of the parafascicular nucleus medial to the habenulo-peduncular tract are less severe than in the lateral part. While the latter had undergone total cell atrophy many cells persisted in the medial part. This difference in the degree of reaction between the medial and lateral parts has been recognized in all the other experiments in which this nucleus showed changes. The changes in the reticular nucleus are confined to its anterior third, and are more marked in the medial half; in this portion of the nucleus there is marked gliosis, shrinkage of the intercellular spaces with slight cell loss, and pallor and swelling of the remaining cells.

This experiment and the others in this group confirm the findings of Rose & Woolsey (1943) that the cells of the midline and intralaminar nuclei, as well as those of the main nuclei, will undergo degeneration following extra-thalamic lesions. Furthermore, as these authors have pointed out, a striking feature of the degeneration is the manner in which it stops abruptly at the midline after a unilateral lesion. (Pl. 1, fig. 2). An additional finding is that the anterior and posterior paraventricular



nuclei do degenerate along with the other dorsal thalamic elements. In this connexion it may be noted that Walker (1936) has described degenerative changes in the posterior paraventricular nucleus in one experiment (his case 3) in which the preoptic and anterior hypothalamic areas were damaged.



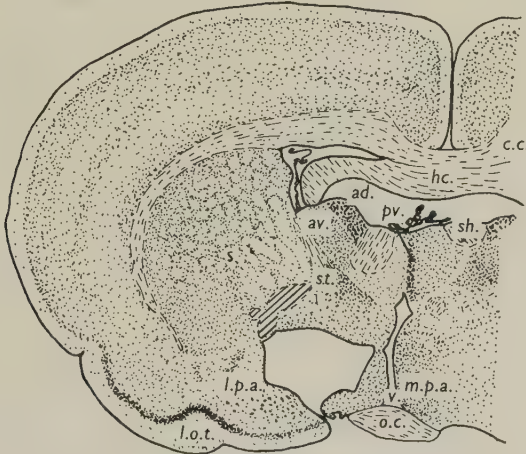
Text-fig. 2. The extent of the resulting degeneration in the thalamus in Exp. R6 (indicated by hatching).

In Exp. R7 the lesion was practically identical with that described for R6 except that there is virtually no direct damage to the amygdaloid nuclei, the medial preoptic area or the hypothalamus. The lateral preoptic area and the adjacent part of the internal capsule are, however, severely damaged. The damage to the internal capsule stops at the level of the rostral end of the thalamus, so that even if the fibre connexions of the amygdala are directed medially only the most anterior would be involved. Since the resulting degeneration is also identical with that described in the previous experiment, it may be inferred that the fibres from the midline and intralaminar nuclei neither terminate in nor traverse the anterior hypothalamus and the medial preoptic area; the anterior part of the amygdala, however, cannot be definitely excluded.

In the third experiment of this group, R10, the only essential difference in the

extent of the lesion from that of R6 is the absence of direct involvement of the septum and medial preoptic area. This suggests that if the septum does receive fibres directly from the midline and intralaminar nuclei these must pass through the internal capsule and not forwards close to the midline.

*Group II.* In the second group of three experiments the midline and intralaminar nuclei degenerated while, with few exceptions, the adjacent main nuclei remained intact. In R29 the electrode track enters the anterior cingular cortex of one side and passes backwards, downwards and medially through the corpus callosum into the lateral septal nucleus of the opposite side. Though the direct damage to this

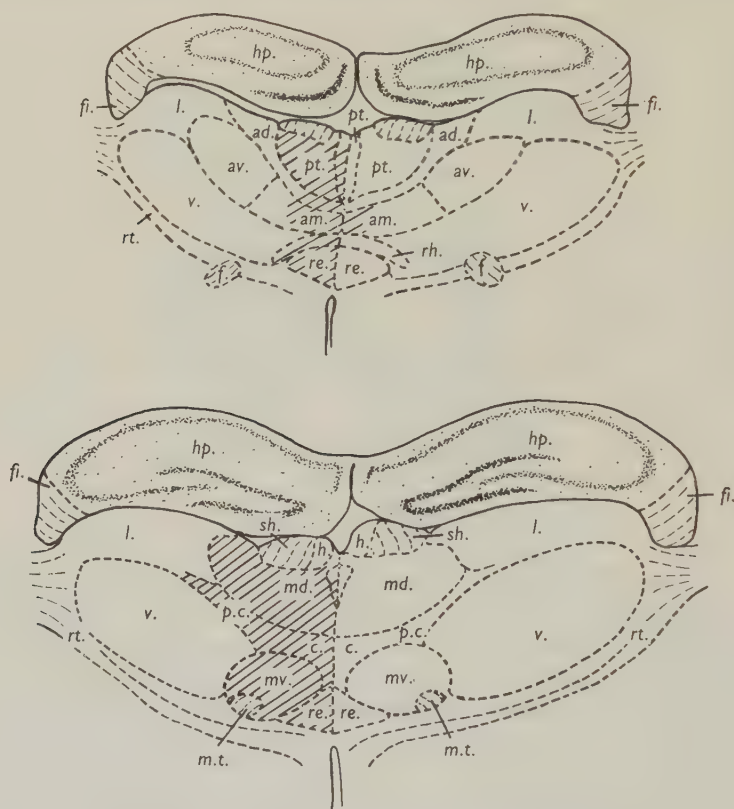


Text-fig. 3. Site of lesion in Exp. R29.

nucleus is not extensive, being restricted to the narrow electrode track, the septum of this side is greatly shrunken and the cells compacted together following the loss of neuropil. The large-celled medial septal nucleus and the vertical limb of the diagonal band nucleus are not directly involved, but the majority of their cells have undergone secondary degeneration. The track continues through the lateral septal nucleus towards the inferior angle of the lateral ventricle into the medial preoptic area. The principal damage is in this and the lateral preoptic area, the posterior halves of which are completely destroyed (Text-fig. 3). The lesion extends posteriorly into the lateral hypothalamic area as far back as the level of the middle of the supraoptic nucleus. The posterior margin of the nucleus accumbens, the ventromedial part of the striatum and the ventral margin of the pallidum have been slightly damaged. The ventral third of the internal capsule has been destroyed at, and for a short distance behind, the anterior end of the thalamus. Severe gliosis can be seen extending posteriorly from this into the inferior thalamic radiation (both in the internal capsule and in the medial part of the thalamus of the affected side).

All the midline and intralaminar nuclei of the thalamus of the affected side, with the exception of the parafascicular, have undergone almost complete retrograde degeneration (Text-fig. 4). The degenerated nuclei are: the anterior and posterior

paraventricular, parataenial, rhomboid, reuniens, medial ventral, centralis, paracentralis, inter-antero-dorsal, inter-antero-medial and inter-medio-dorsal. This degeneration consists of marked cell loss, with only occasional pale shrunken cells remaining, and severe gliosis (Pl. 2, fig. 3). The only main nuclei which have degenerated are the entire antero-medial, the medio-dorsal and the medial third of the ventral anterior of the affected side; on the normal side there is a small localized area of degeneration in the antero-medial nucleus. With the exception of the lower medial part of its rostral end the reticular nucleus shows no change. In the affected



Text-fig. 4. Thalamic degeneration in Exp. R 29.

parts of the reticular and ventral anterior nuclei there is definite gliosis continuous with that in the inferior thalamic radiation together with pallor and swelling of the cells; that this gliosis is due to the degeneration of fibres passing through these nuclei is confirmed by the protargol preparations. In these the loss of fibres in the inferior thalamic radiation (both in the internal capsule and thalamus) is quite distinct. The changes in the reticular nucleus, found here and in group I, are in accord with the observations of Combs (1949) following hemidecortications in the rat and may be considered to be in agreement with the concept of Rose (1950) in regard to the projection of this nucleus.



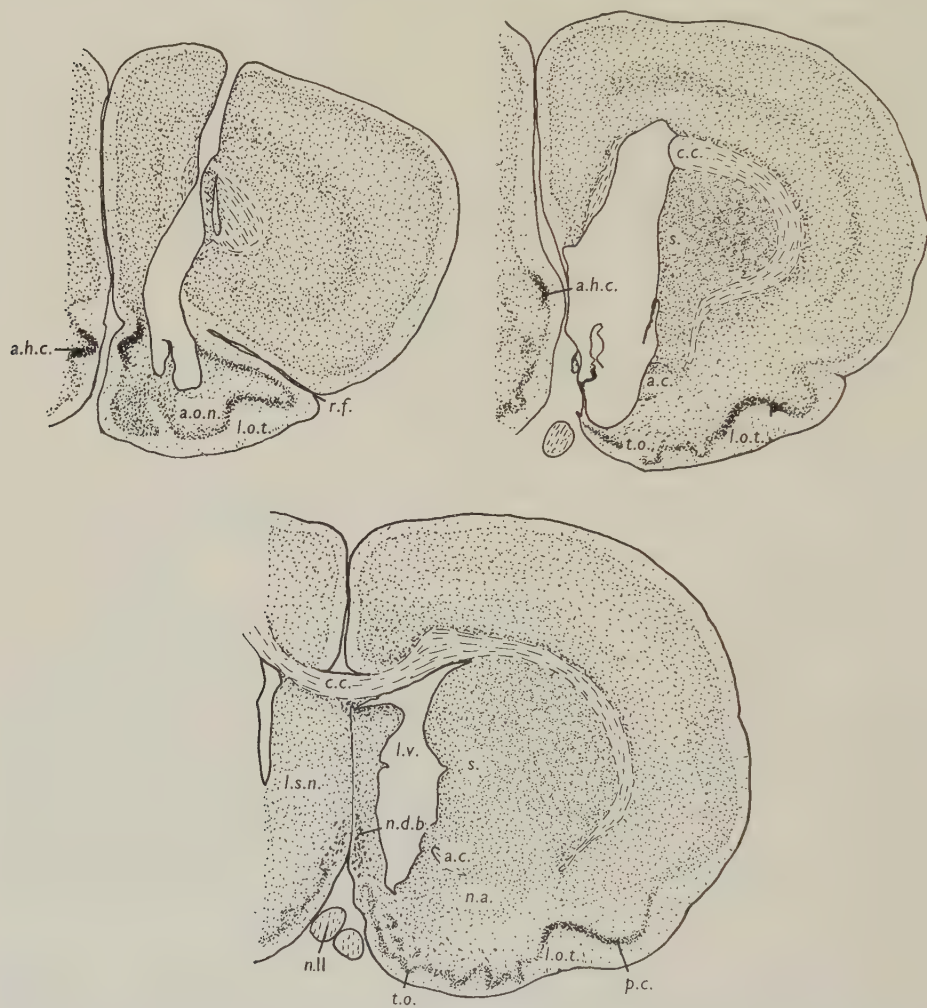
This experiment indicates that the fibres from the medial cell groups of the thalamus project by way of the inferior thalamic radiation in close relation to the nucleus ventralis anterior and the lateral preoptic area. In the second experiment of this group (P3) the lesion was very similar except for the additional involvement of the anterior end of the thalamus including the nucleus ventralis anterior, the medial half of the reticular nucleus, the antero-medial and the ventro-medial part of the antero-ventral nucleus and the rostral part of the nucleus reuniens. The resulting degeneration in the midline and intralaminar nuclei is the same. Exp. RF1 is again very similar except that the lateral preoptic area is only damaged in its dorsal part, and that above this the lesion extends into the caudal part of the nucleus accumbens and the medial half of the striatum and pallidum with involvement of the extreme rostral end of the internal capsule in front of the thalamus. Medially the lesion has destroyed the posterior two-thirds of the septum of that side and the medial septal nucleus and postero-medial part of the lateral septal nucleus of the opposite side. The thalamic degeneration is almost identical with that in R29. The significance of this lesion is that it demonstrates quite clearly that the fibres from the midline and intralaminar nuclei project through the rostro-ventral part of the internal capsule into (or through) the ventro-medial parts of the striatum and pallidum and the dorsal part of the preoptic area. It also confirms the conclusion drawn from the first group of experiments that the septum and medial preoptic areas are not directly connected with this projection. On the other hand, also, it is unlikely that very many of the fibres from these nuclei terminate in the lateral preoptic area as most of the midline nuclei undergo degeneration after lesions placed at more rostral levels. The nineteen experiments in which this has occurred constitute the third group.

*Group III.* In R16 the lesion (Text-fig. 5) is first seen at the level of the accessory olfactory bulb; here the frontal pole of the hemisphere, the pars dorsalis, pars lateralis and probably the fibres from the pars ventralis of the anterior olfactory nucleus are destroyed. In their anterior parts the entire nucleus accumbens and the medial half of the caudate nucleus have been destroyed but more posteriorly only their medial margins are encroached upon. The medial third of the rostral part of the olfactory tubercle has been destroyed and the fibres from the medial half have probably been interrupted. The anterior hippocampal cortex and the lateral two-thirds of the anterior half of the lateral septal nucleus have been ablated before the lesion diminishes rapidly to end in front of the level of the large-celled medial septal nucleus.

The nucleus reuniens and the rhomboid nucleus show almost total cell atrophy except for a few pale, shrunken cells persisting in the medial part of the former (Text-fig. 6). The parataenial nucleus as a whole has shrunk to approximately half of its normal cross-sectional area and shows marked cell loss but little gliosis (Pl. 2, fig. 4). In some sections the anterior paraventricular nucleus appears to have undergone some cell loss particularly in its caudal half. The medial ventral nucleus is severely degenerated. In the nucleus centralis and paracentralis a slight cell loss has occurred, and some of the remaining cells are swollen and paler staining. The only main nuclei affected are the antero-medial and the anterior half of the medio-dorsal in both of which there is a moderate cell loss with shrinkage and pallor of

a proportion of the remaining cells. It may be noted that of the medial cell groups only the parafascicular and posterior paraventricular nuclei show no change and that the reticular nucleus also showed no cellular degeneration.

From this experiment it may be inferred that the midline and intralaminar nuclei project (at least in part) to the structures abutting on the medial surface of the

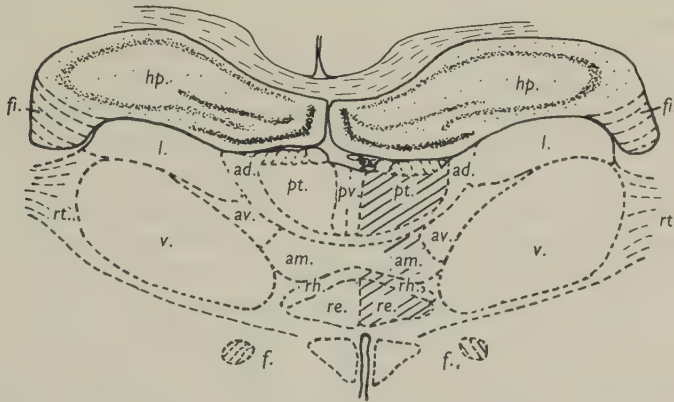


Text-fig. 5. Site of lesion in Exp. R16.

hemisphere in front of the septum. The damage to the lateral septal nucleus does not seem to be significant in view of the other experiments which indicate that the septum has no direct connexion with the dorsal thalamus (cf. R30 and R31). The only structures which are appreciably damaged are the medial half of the anterior end of the striatum, the neocortex on the medial surface of the hemisphere and the medial part of the olfactory tubercle. This is also the first experiment in this series

in which degeneration of these nuclei has occurred without involvement of the lateral preoptic area, the internal capsule or the pallidum.

In the other experiments of this group the lesions, though smaller, involve more or less the same structures at approximately the same level. In all of these some degenerative change has taken place in one or more of the midline and intralaminar nuclei. In some (R18, 26, 27) the nucleus reuniens, the medial ventral and the rhomboid nucleus together showed these changes (Pl. 1, fig. 1). An example of this type is R18 in which the electrode has entered the dorsal aspect of the frontal pole and passed downwards, medially and backwards to destroy the cortex on the ventral



Text-fig. 6. Degeneration in the midline and intralaminar nuclei in Exp. R16.

half of the medial surface of the hemisphere as far back as the anterior hippocampal cortex and the medial surface of the caudate nucleus and the nucleus accumbens. The dorsal and medial parts of the anterior olfactory nucleus and the rostromedial margin of the olfactory tubercle have also been involved. It should be stressed that the lesion does not extend behind the level of the genu of the corpus callosum.

In all the other experiments of this group only the medial ventral nucleus and nucleus reuniens (either alone or together) degenerated. Although most of the lesions have involved, to some extent at least, the subjacent white matter, Lashley's (1941) observation that the medial ventral nucleus projects to the antero-lateral aspect of the frontal lobe and the nucleus reuniens to the medial surface is confirmed by our material. The precise projection of the other midline nuclei has not been determined because of the concomitant involvement of cortical and subcortical structures. This is being studied in the brains of larger animals, as in the rat we have found it difficult to place lesions on the medial surface of the hemisphere strictly confined to the cortex and are therefore unable to say whether the fibres have been damaged close to their termination or interrupted in their course.

In so far as we have been able to work out the spatial organization of the projection of the midline nuclei our findings are in accord with those of Droogleever-Fortuyn (1950) and earlier workers. Thus the medial ventral projects most rostrally, and then at successively caudal levels the nucleus reuniens, the rhomboid and the parataenial nucleus. Further, from a correlation of the extent of degeneration in



these restricted lesions with the more extensive degeneration found in the previous experiments, it seems that whereas the midline nuclei are connected with the cortex of the medial surface of the hemisphere, the intralaminar nuclei probably project to the striatum, which would agree with the physiological findings of Jasper and his co-workers (1949, 1950). On this ground it is suggested that in the diffuse projection system two inter-related mechanisms are probably involved—one with a direct projection to the medial surface of the hemisphere and one to the striatum. Droogleever-Fortuyn (1950) divided the nucleus reuniens into two elements, a paramedian nucleus anteriorly and a nucleus submedius posteriorly; he also states that these have different projection fields. In some of our experiments we have noticed a difference in the degree of reaction in the anterior and posterior portions of the nucleus which lends support to this subdivision. It may be noted that in the lesions which damaged the antero-medial part only of the striatum no degeneration was found in the parafascicular nucleus; similarly, no change was observed in the paraventricular nuclei in the experiments of this group.

*Group IV.* The six experiments in the fourth group eliminate the possibility that the midline and intralaminar nuclei project directly to the anterior olfactory nucleus, the olfactory tubercle, the main part of the septum and the medial preoptic area. In three experiments (R3, R5 and R13) practically the entire anterior olfactory nucleus has been damaged without involvement of any other structure. In none of these could retrograde cell degeneration be detected in the thalamus.

In two experiments the septum has been considerably damaged without significant degeneration in the thalamus. In R30 the electrode enters the cingular gyrus of the left side just anterior to the genu of the corpus callosum and, partially destroying the genu and the dorsal part of the anterior hippocampal cortex of both sides, it continues into the septum. The extreme rostral end of the lateral septal nucleus (just at the level of the fusion of the septum of either side) is spared on the left side but is damaged in its dorsal half on the right. Behind this the entire septum of the right side has been ablated but on the left the ventral third of the lateral septal nucleus, together with the septofimbrial nucleus, has been spared. The dorsal two-thirds of the medial preoptic area in front of the anterior commissure has been severely damaged on the right side.

The only change in the thalamus is in the rostral half of the nucleus reuniens of both sides and in the antero-medial and the medial half of the antero-ventral nuclei of the left side; all of these show marked cell loss. The reaction in the nucleus reuniens cannot be attributed to the septal damage, as the experiments of the previous group have shown that the projection field of this nucleus is situated anterior to the genu of the corpus callosum. The essential difference between this and Exp. R31 is that in the latter the medial preoptic area was more extensively damaged. The thalamic degeneration, however, is confined to the antero-medial nucleus.

The last experiment of this group to be described is R12 in which only the medial half of the rostral two-thirds of the olfactory tubercle has been destroyed with no resulting degeneration in the thalamus. This experiment, taken in conjunction with R16, would tend to exclude the rostro-medial part of the olfactory tubercle as the essential projection area of the midline and intralaminar nuclei.

## CONCLUSIONS

The observations described here have fully confirmed the important findings of Rose & Woolsey (1943, 1949) that the midline and intralaminar nuclei of the thalamus undergo retrograde degeneration following large telencephalic lesions. In addition, in experiments with more localized damage it has been demonstrated that these nuclei project independently of the main nuclei by way of the inferior thalamic radiation as was suggested by Droogleever-Fortuyn (1950). In this second group all the midline and intralaminar nuclei, except the parafascicular, degenerated following lesions in which the main damage was localized to the lateral preoptic area and the adjacent parts of the striatum and pallidum; of the main nuclei only the medial dorsal and antero-medial nuclei were affected. Most of this degeneration was the result of interruption of fibres passing forwards through this area as these nuclei also degenerated following lesions rostral to the level of the genu of the corpus callosum. When most of the cortex of the medial surface of the hemisphere back to this level together with the adjacent portion of the striatum is destroyed, the only nuclei of the midline and intralaminar group which did not degenerate were the parafascicular and paraventricular nuclei. The single experiment of Lashley (1941) and Exp. 6 of Droogleever-Fortuyn (1950) are comparable to this. Although smaller lesions involving these structures resulted in degeneration of only some of these nuclei it was not possible to localize the projection area of individual nuclei as in each case both cortical and subcortical structures were involved. There is, however, some evidence of a rostro-caudal spatial organization within this projection. It is important to note that in all the hemidecortications in which the midline and intralaminar nuclei were preserved (Walker, 1938*a, b*; Powell, 1952), it was these structures which were largely spared. The experiments of the final group, taken together with some of those with the more extensive lesions, almost certainly exclude most of the septum, olfactory tubercle, the medial preoptic area, amygdala and anterior olfactory nucleus from receiving fibres directly from the midline and intralaminar nuclei. It may be concluded that the midline and intralaminar nuclei of the thalamus of the rat have an independent projection by way of the inferior thalamic radiation to the cortex of the medial surface of the hemisphere and the striatum, and that they do undergo retrograde cell degeneration after lesions involving these structures.

## SUMMARY

1. The projection of the midline and intralaminar nuclei of the thalamus has been studied in the rat by the method of retrograde cell degeneration.
2. Following extensive lesions of the rostral telencephalon all dorsal thalamic elements including these nuclei degenerate.
3. These nuclei degenerate alone after lesions in which the main damage is localized to the lateral preoptic area and the adjacent parts of the striatum and pallidum.
4. Degeneration has occurred in most of these nuclei following destruction of the head of the striatum and the cortex of the medial surface of the hemisphere in front of the genu of the corpus callosum.

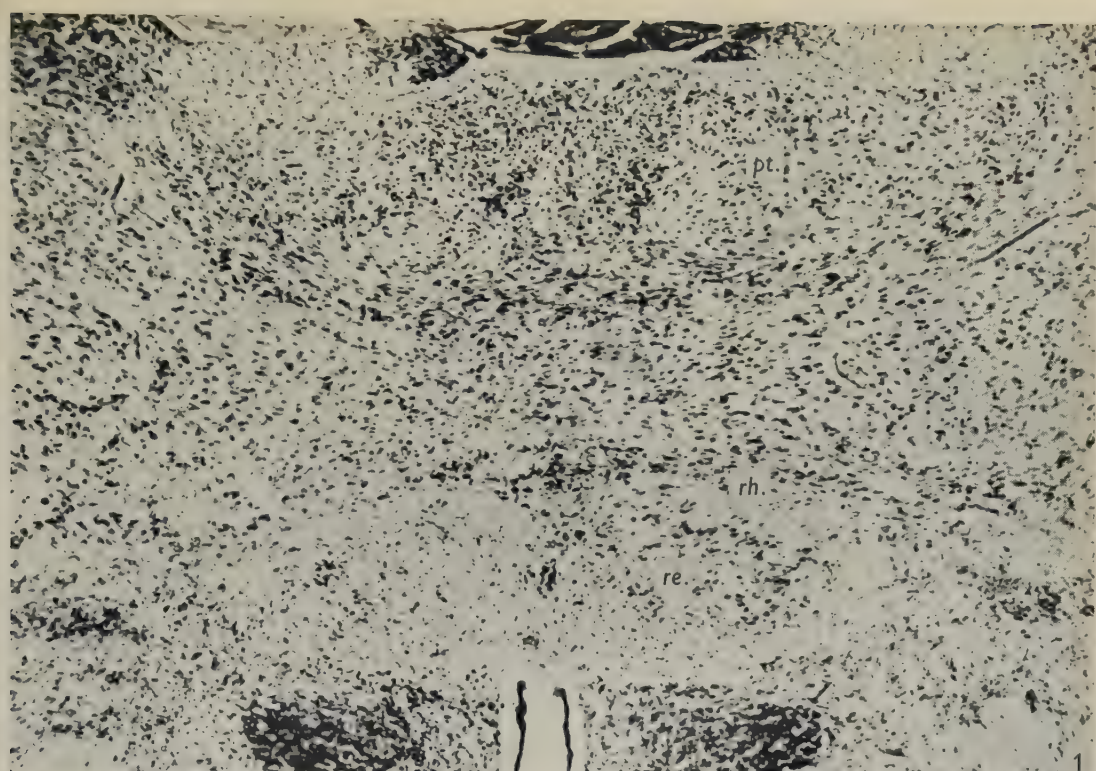
5. It is probable that most of the septum, the medial preoptic area, the amygdala, the olfactory tubercle and the anterior olfactory nucleus are not directly connected with these nuclei.

This work was done while one of us (T.P.S.P.) held a Medical Research Council Fellowship in Clinical Research. We wish to acknowledge the valuable technical assistance of Mr Michael Lindsey.

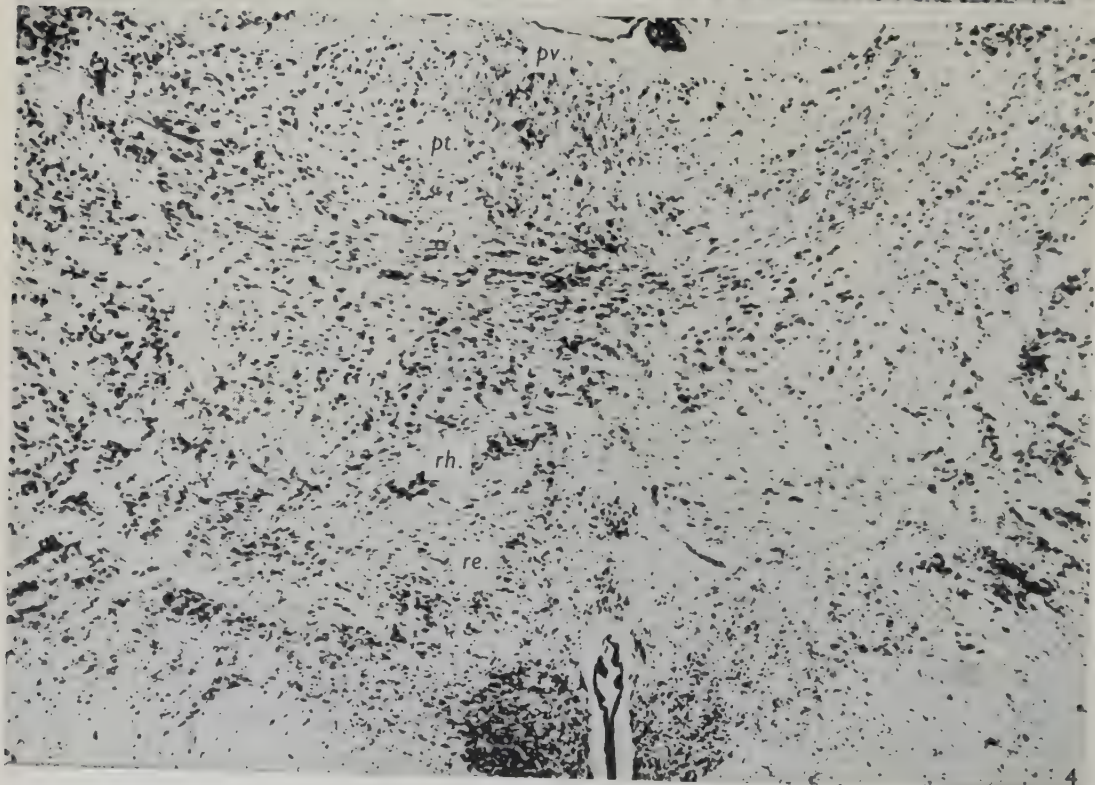
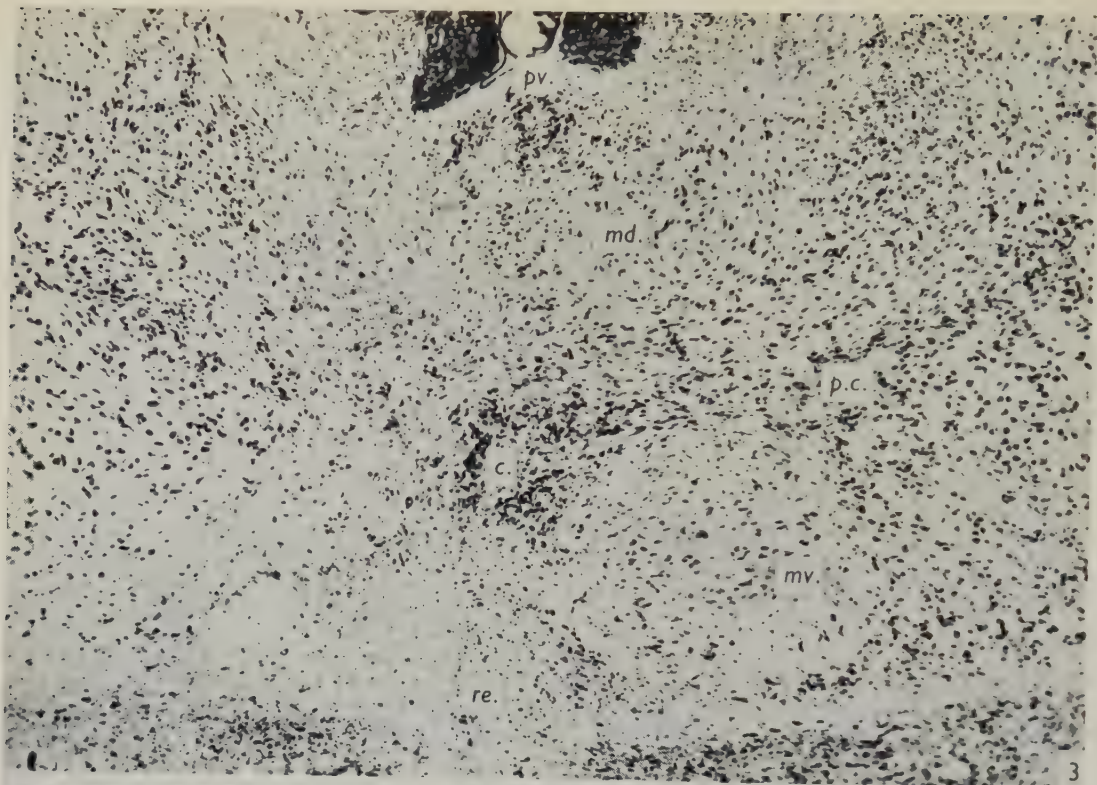
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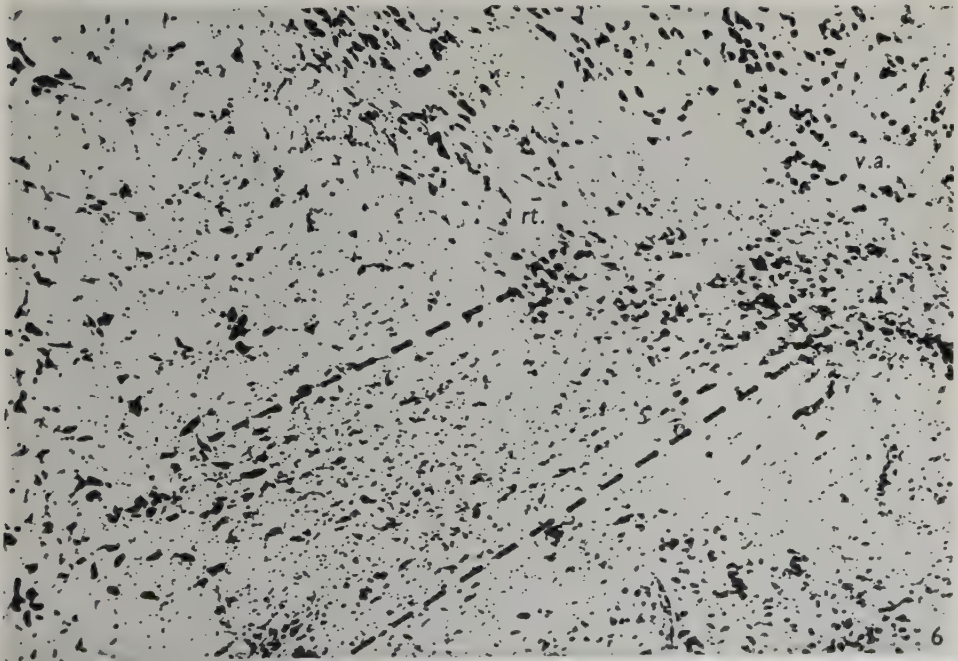
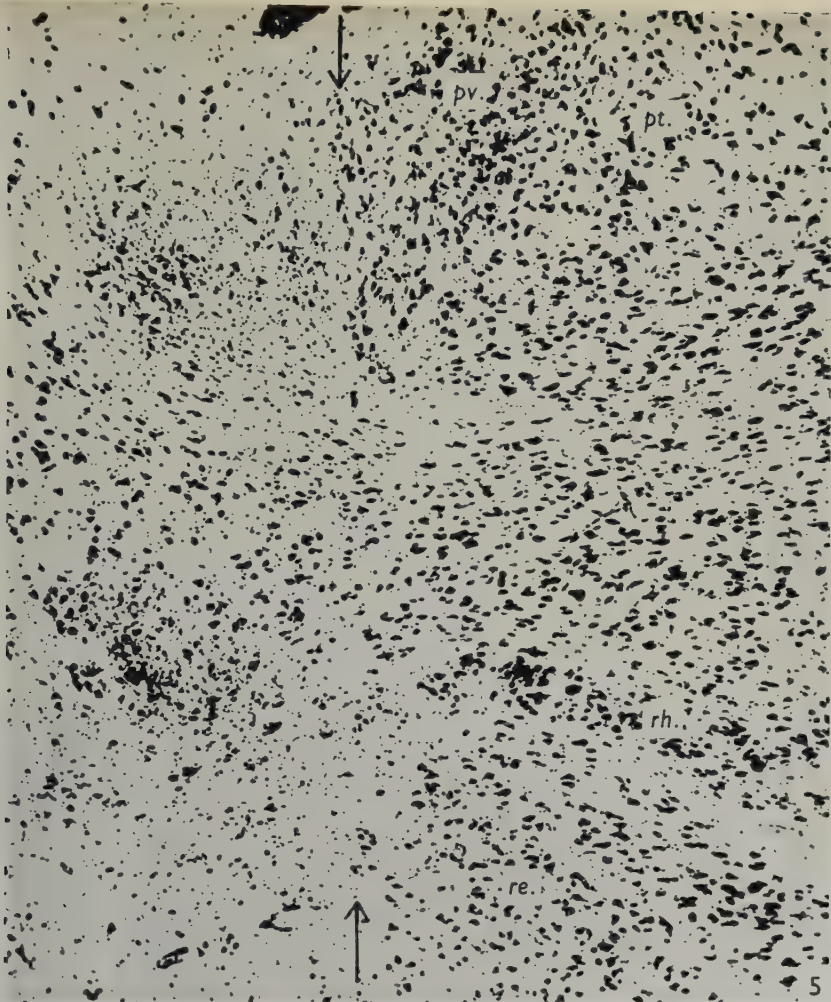
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## EXPLANATION OF PLATES

All the sections were stained with methylene blue. The magnification of figures 1-4 is  $\times 58$ , and of figures 5 and 6 is  $\times 92$ .

## PLATE 1

Fig. 1. Photomicrograph showing degeneration in the nucleus reuniens and lateral part of the rhomboid nucleus of the right side in Exp. R 26.

Fig. 2. To show unilateral degeneration of almost all dorsal thalamic elements in Exp. R 6.

## PLATE 2

Fig. 3. To show degeneration of the midline and intralaminar nuclei in Exp. R 29. Observe cell loss and gliosis in posterior paraventricular nucleus, nucleus centralis, paracentralis, medial dorsal and medial ventral and nucleus reuniens.

Fig. 4. To show degeneration of parataenial, inter-antero-dorsal, inter-antero-medial, rhomboid and nucleus reuniens in Exp. R 16.

## PLATE 3

Fig. 5. To show degeneration in parataenial, antero-medial, rhomboid and nucleus reuniens in Exp. R 29. (The arrows indicate the midline.)

Fig. 6. To show gliosis (outlined) in the inferior thalamic radiation in Exp. R 29.

## ABBREVIATIONS

<i>a.c.</i>	anterior commissure	<i>m.s.n.</i>	medial septal nucleus
<i>ad.</i>	antero-dorsal nucleus	<i>m.t.</i>	mamillo-thalamic tract
<i>a.h.c.</i>	anterior hippocampal cortex	<i>mv.</i>	medioventral nucleus
<i>am.</i>	antero-medial nucleus	<i>n.a.</i>	nucleus accumbens
<i>a.o.n.</i>	anterior olfactory nucleus	<i>n.d.b.</i>	nucleus of diagonal band
<i>av.</i>	antero-ventral nucleus	<i>n. II</i>	optic nerves
<i>c.</i>	nucleus centralis	<i>o.c.</i>	optic chiasma
<i>c.c.</i>	corpus callosum	<i>p.c.</i>	paraventricular nucleus
<i>f.</i>	fornix	<i>pt.</i>	parataenial nucleus
<i>fi.</i>	fimbria	<i>pv.</i>	anterior paraventricular nucleus
<i>h.</i>	medial habenular nucleus	<i>re.</i>	nucleus reuniens
<i>h.c.</i>	hippocampal commissure	<i>r.f.</i>	rhinal fissure
<i>hp.</i>	hippocampus	<i>rh.</i>	rhomboid nucleus
<i>l.</i>	lateral nucleus	<i>rt.</i>	reticular nucleus
<i>l.o.t.</i>	lateral olfactory tract	<i>s.</i>	striatum
<i>l.p.a.</i>	lateral preoptic area	<i>s.h.</i>	stria habenularis
<i>l.s.n.</i>	lateral septal nucleus	<i>s.t.</i>	stria terminalis
<i>lv.</i>	lateral ventricle	<i>t.o.</i>	olfactory tubercle
<i>md.</i>	mediodorsal nucleus	<i>v.</i>	third ventricle
<i>m.p.a.</i>	medial preoptic area	<i>v.a.</i>	ventral anterior nucleus

# HISTOLOGICAL AND FUNCTIONAL STUDIES ON THE FIBRE COMPOSITION OF THE VAGUS NERVE OF THE RABBIT

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The study was undertaken with the following main objects in view:

(1) To obtain quantitative information on the total number of medullated and non-medullated fibres contained in the cervical, thoracic and abdominal portions of the vagus.

(2) To differentiate between the afferent and efferent components of the nerve as regards fibre number and the range of fibre diameter contained in each functional group.

(3) To determine the number of adventitial fibres in the rabbit's vagus, derived from the sympathetic trunk and other sources.

In the cat a number of investigations have been made on the distribution of sensory and motor fibres within the vagus and its branches (Ranson, Foley & Alpert, 1933; Heinbecker & O'Leary, 1933*a, b*; Dubois & Foley, 1936; Foley & Dubois, 1937; Daly & Evans, 1953). These authors relied mainly on studies of the histological changes produced in the nerve by chronic degenerative section of the vagus cut either intracranially or extracranially between the nodose ganglion and the base of the skull. These papers are chiefly concerned with the cervical vagus nerve and its branches and contain comparatively little information on the thoracic and abdominal extent of the nerve. In three of these investigations some differential sensory and motor counts were made. Foley & Dubois (1937) determined the proportion of sensory and motor fibres in the vagus trunk above the nodose ganglion in the cat, and reported that the sensory group constituted 65-80% of the total. In an earlier paper (Dubois & Foley, 1936) they estimated the proportion of the sensory and motor fibres in some of the cervical branches of the vagus. Daly & Evans (1953) give data on the numbers of sensory and motor fibres of the medullated and non-medullated groups in the cat's thoracic vagus trunk and in its cardiac and bronchial branches.

As far as we are aware, no quantitative analysis of the abdominal vagus nerve, differentiating its fibres into sensory and motor components, has so far been made. Accordingly, in the present study, attention has been concentrated on the abdominal vagus, although additional information has been accumulated about the cervical and thoracic components.

## METHODS

*Histological procedures.* The normal and experimental material was obtained from adult rabbits of varying breeds. The relevant portions of the vagus nerve trunk and its branches were removed from animals anaesthetized with pentobarbitone sodium (Nembutal) (30 mg./kg. body weight, intravenously) supplemented with ether.



For study of myelinated fibres the nerves were placed on cardboard frames in a lightly stretched condition and fixed for 24 hr. in Flemming's fluid (1% chromic acid, 15 ml.; 2% osmic acid, 4 ml.; glacial acetic acid, 1 drop). The nerves were sectioned transversely ( $5\mu$  thickness) after paraffin embedding and stained by a modified Weigert technique described by Gutmann & Sanders (1943). The sections were photographed at magnification of  $\times 750$  directly on bromide paper, and the outside diameter of the myelinated fibres measured and classified into  $2\mu$  groups. Measurements were made by means of a Perspex sheet on which were imprinted circles in a sequence of increasing diameters (1.5, 3, 4.5 mm. etc). As each fibre was measured it was pricked with a needle connected to an electric counter. The possible errors in such measurements have been discussed in the works of Gutmann & Sanders (1943), Aitken, Sharman & Young (1947), Sanders (1947) and Evans & Vizoso (1951). The effects of variations in technique were minimized, as far as possible, in the present studies by taking the series of nerves to be compared through the stages of fixation, staining and photography together.

Greatest success in the staining of non-myelinated fibres was obtained with the Ranson pyridine-silver method (Ranson & Davenport, 1931). On completion of the staining process the spinal cord, with its contained nerve, was embedded in paraffin and sectioned transversely at  $2-3\mu$  thickness. The best results were obtained with alcohol ammonia solution (96% alcohol, 99 ml., 0.880 ammonia, 1 ml.) as fixative, although this produced extensive shrinkage of the axons. In satisfactory preparations the non-myelinated and small myelinated axons were stained black with sharp discrete outlines, whilst the large myelinated fibres often appeared light brown.

Estimates of the total number of axons in pyridine-silver preparations were made by a sampling method (Evans & Murray, 1953). The area of nerve sampled varied from 17 to 33% of the total cross-sectional area of each nerve, this proportion depending upon the evenness of distribution of the axons.

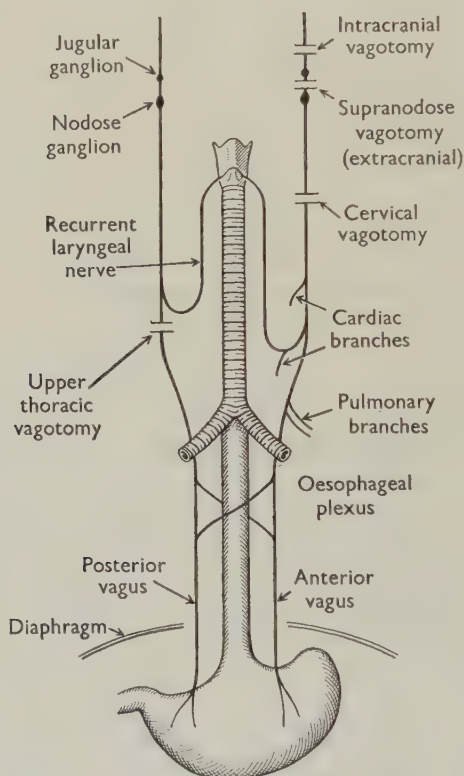
*Nerve sections.* Four series of experiments involving degenerative section of the vagus were carried out under surgical anaesthesia with full aseptic precautions (Text-fig. 1).

(1) Intracranial section of the left vagal rootlets, performed successfully in two rabbits. These rootlets were approached by a dorsal mid-line incision extending from the level of the external occipital protuberance caudally for a distance of 6 cm. The medulla was retracted gently to the right and the vago-accessory rootlets close to the medulla cut under direct vision. Care was taken to cut the most cranial rootlets (including those of the glossopharyngeal nerve) which curve laterally along the surface of the anterior margin of the jugular foramen. After a post-operative survival period of 17 and 25 days respectively, the animals were brought to biopsy, at the termination of which the skull was re-opened post-mortem and an examination for intact rootlets made.

(2) Unilateral supranodose (extracranial) vagotomy performed in eight animals. The cervical vagus nerve was cut between the base of the skull and upper pole of the ganglion nodosum, in each case 2 mm. or more above the upper pole of the ganglion. Care was taken not to injure the ganglion, which was brought ventrally and placed on the side of the pharynx to minimize the possibility of fibres regenerating into the distal stump.

(3) Right upper thoracic vagotomy followed 21 days later by left supranodose vagotomy. In this set of two animals 1 cm. of the right vagus immediately below the origin of the recurrent laryngeal nerve was removed.

(4) Right upper thoracic vagotomy followed 21–30 days later by resection of 1 cm. of the left vagus at the mid-cervical level. This was performed in three animals.



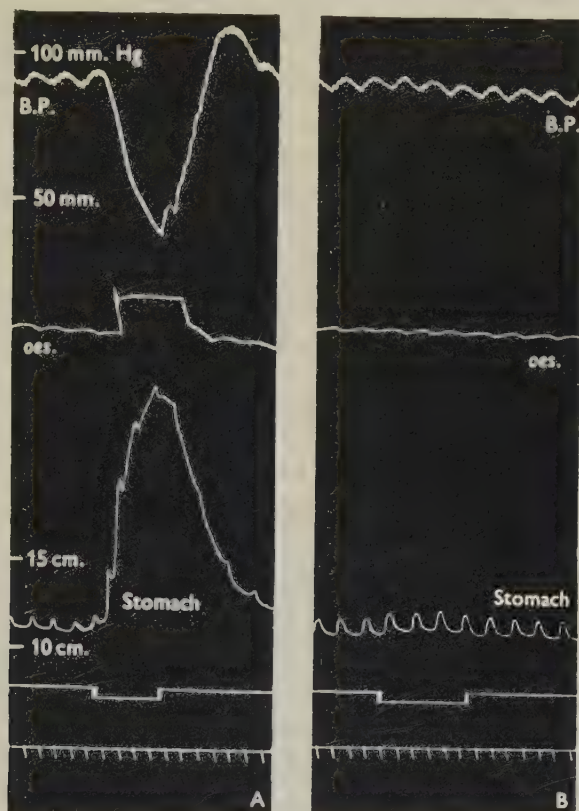
Text-fig. 1. Diagram showing the levels of the experimental lesions.

The animals were brought to biopsy 17–30 days after the latest operation, thus allowing time for fibres cut off from their cell bodies to degenerate. At this stage, in the series of animals subjected to intracranial or unilateral supranodose vagotomy, the cervical vagus on the treated side was stimulated electrically and the effects on the blood pressure, heart rate and intraoesophageal and intragastric pressures recorded. Intraoesophageal and intragastric pressures were recorded by placing an air-filled balloon connected to a rubber tambour recorder in the lumen of the viscus (Evans & Murray, 1953). On the operated side the ganglion nodosum and the portion of nerve trunk cranial to it were subsequently removed and fixed in Bouin's fluid. The ganglia were sectioned longitudinally and stained by the Bodian, Nissl or Heidenhain's azo-carmin methods.

## RESULTS

*Functional localization in the nodose and jugular ganglia*

In order to determine whether motor nerve cells exist in the rabbit's nodose or jugular ganglia, observations were made on the effects of stimulation of the operated and control vagi upon the blood pressure, heart rate and intraoesophageal and intragastric pressures after chronic intracranial or supranodose section of the vagus. The effects obtained in typical members of these two series are shown in



Text-fig. 2. Records showing the effects on the blood pressure, intraoesophageal (*oes.*) and intragastric pressure of stimulation of the caudal cut end of the normal (A) and treated (B) cervical vagus nerves. Left supranodose (extracranial) vagotomy performed 21 days' previously. Time intervals on the tracings equal 1 sec.

Text-fig. 2. On the normal side the usual fall in blood pressure and slowing of the heart were obtained, but in all animals stimulation of the operated nerve produced no effect in spite of wide variation in stimulus voltage (10–25 V.), frequency (40–200 cyc./sec.) and pulse duration (0.01–10 msec.). Stimulation of the cervical vagus on the operated side failed to produce pressure changes in the oesophagus in all the animals tested. It was also found that chronic intracranial or supranodose (extracranial) vagotomy invariably produced degeneration of the vagal efferent



fibres to the stomach, as shown by lack of intra-lumen pressure change with stimulation of the cervical vagus on the operated side. By contrast stimulation of the peripheral cut end of the normal cervical vagus produced sharp increase in intragastric pressure. Evans & Murray (1953) found that this effect occurred invariably with stimulation of the right or left normal vagus in a control series of thirty-six rabbits.

In the present investigation serial longitudinal sections have been prepared of normal nerves consisting of the vagal rootlets, the nodose and jugular ganglia and of the interganglionic nerve trunk. Such sections show a few scattered rows of ganglion cells amongst the nerve fibres in the interganglionic nerve trunk. However, although no counts of such cells have been made, their number is probably insignificant compared with the number contained in the two ganglia. No nerve cells were found in the vagal roots cranial to the upper pole of the jugular ganglion.

In all the animals subjected to supranodose (extracranial) interruption of the nerve, serial longitudinal sections were prepared of the nodose ganglion and the nerve trunk cranial to it, including the terminal neuroma. Such sections show that the nerve cells in the upper pole of the nodose ganglion and the majority of those in the supraganglionic nerve trunk are normal in appearance (Pl. 1, fig. 5). Evidently those ganglion cells situated near the level of interruption of the nerve degenerate, but these are comparatively few in number and these presumably distribute their fibres mainly in the superior laryngeal and cardiac depressor nerves (Molhant, 1913). These two nerves have not been investigated in the present study.

## HISTOLOGICAL FINDINGS

### *The cervical vagus*

Table 1 gives counts of total fibre number (medullated and non-medullated) in normal vagi and in nerves treated by chronic supranodose (extracranial) or intracranial vagotomy. The number of myelinated fibres and their size-frequency distribution are shown in Table 2. All specimens were taken at the lower cervical levels, where the vagus travels for several centimetres without branching.

Table 1. *Estimates of the total number of axons (medullated and non-medullated) in the cervical vagus nerve*

(All the operated specimens were obtained from the left side.)

Normal				Operated			
Left		Right		Supranodose vagotomy		Intracranial vagotomy	
Animal and specimen	Estimate	Animal and specimen	Estimate	Animal and specimen	Estimate	Animal and specimen	Estimate
19a	26,529	22a	23,385	24a	17,761	26a	18,578
20a	24,328	23a	20,486	25a	17,539	27a	18,683
21a	23,354	20b	20,712	—	—	—	—

Comparison of the values given in Tables 1 and 2 shows that the normal cervical vagus of the rabbit is a predominantly non-medullated nerve, only about 12.6% (mean value) of the total fibre content being medullated. In transverse section of normal nerves stained by Weigert method (Pl. 1, fig. 1) the medullated fibres are

seen to be unevenly distributed throughout the nerve. In high-power view of the nerve stained by the pyridine-silver technique the areas between the medullated fibres are shown to contain densely packed groups of non-medullated axons (Pl. 1, fig. 3).

Table 2. *Size-frequency distribution of medullated fibres in the vagus at the lower cervical levels*

(L (left) and R (right) denote the side from which the nerve was taken.)

Animal and specimen		No. of fibres in diameter groups of $2\mu$							Total
		0-2	2-4	4-6	6-8	8-10	10-12	12-14	
(a) Normal									
1a	R	62	1483	549	312	63	66	20	2555
2a	R	105	1832	560	283	181	96	30	3077
3a	L	66	1029	685	234	184	91	21	2301
4a	L	40	1312	1154	466	242	126	66	3406
7a	L	41	876	1605	353	168	117	58	3220
(b) Supranodose vagotomy									
6a	L	16	751	637	161	148	9	0	1722
8a	L	29	549	656	130	74	9	0	1447
9a	L	54	586	884	226	122	20	0	1892
10a	L	47	904	993	139	112	24	0	2219
11a	L	36	925	1219	462	190	41	0	2873
15a	L	34	773	727	335	94	3	0	1966
(c) Intracranial vagotomy									
26b	L	14	681	1143	365	137	41	0	2381
27b	L	33	614	796	241	183	47	2	1916

The two procedures of supranodose (extracranial) and of intracranial vagotomy produced similar degenerative changes in the cervical vagus nerves. These effects are illustrated in Tables 1 and 2 and in Pl. 1, figs. 1, 2, 3 and 4, and may be summarized as follows:

(a) The main degenerative changes are confined to a peripheral segment of the nerve, occupying about a quarter of the total area. In the normal nerve this segment contains predominantly large medullated fibres with some smaller medullated fibres and small groups of non-medullated axons interspersed amongst them. After interruption of the vagus intracranially or extracranially above the nodose ganglion the myelinated fibres in this segment of the nerve completely disappear (Pl. 1, fig. 2). The appearance of this area of almost complete fibre degeneration was a constant finding in the treated nerves.

Examination of the remaining segment of the treated nerves shows little deviation from normal in fibre density both as regards medullated and non-medullated fibres.

(b) Fibre counts show that a proportion of both the medullated and non-medullated groups degenerate. From counts made in pyridine-silver preparations (Table 1), it seems that 20-25% of the total fibres have degenerated and may therefore be considered to be efferent. The degeneration has affected the medullated and non-medullated fibres in roughly the same proportion, although in the case of the supranodose (extracranial) series a somewhat higher proportion (about 31%) of the medullated group have disappeared.

(c) Study of Tables 2 and 3 shows that degeneration of medullated fibres has

affected mainly the large and small diameter groups. Nearly all the 12–14 $\mu$  diameter fibres have degenerated and only about 24% of the 10–12 $\mu$  group have survived. These large medullated fibres, which are contained in a peripheral segment of the normal nerve, represent the motor innervation of the laryngeal muscles and have their nerve cells in the medulla.

Table 3. *Comparison of the mean number of small (0–4 $\mu$ ), medium (4–10 $\mu$ ), and large (10–14 $\mu$ ) diameter medullated fibres in control and operated cervical vagus nerves*

Animal group	0–4 $\mu$	4–10 $\mu$	10–14 $\mu$	Total
Normal	1369	1408	136	2913
Supranodose vagotomy	784	1218	18	2020
Intracranial vagotomy	671	1432	45	2148

Inspection of Tables 2 and 3 also shows that a considerable proportion of the small medullated fibres (2–4 $\mu$ ) become degenerated after intracranial or supranodose section of the vagus. There is a reduction in the number of fibres in these diameter groups from a mean of 1369 in the normal to 784 after supranodose section and 671 after intracranial section of the vagus. This indicates that 40–50% of these small medullated fibres are motor and have their cell bodies in the medulla.

The number of medullated fibres of medium size (4–10 $\mu$ ) is comparatively little affected by supraganglionic section of the vagus. Some reduction in their number was to be expected as some fibres in this diameter range are normally found in the laryngeal bundle of the recurrent laryngeal nerve. All such fibres are motor and have degenerated in the treated nerves. The disappearance of such fibres would account for an average reduction of about 150 in the total within the 4–10 $\mu$  diameter groups in the treated vagi at the lower cervical level. When these motor fibres are excluded it may be seen from the data given in Table 3 that the great majority, and possibly all, of the remaining fibres in the 4–10 $\mu$  range have their cell bodies in the nodose ganglion and are therefore afferent.

#### *The recurrent laryngeal nerve*

At its origin from the vagus the recurrent laryngeal nerve contains two groups of fibres occupying sharply defined areas (Pl. 2, fig. 6). One area contains large heavily medullated fibres and the other small thinly medullated fibres. In its course between the trachea and oesophagus almost all the small fibres are distributed in cardiac, tracheal and oesophageal branches. Consequently the residual part of the nerve entering the larynx consists almost entirely of large fibres (Pl. 2, fig. 7). Measurements of fibre diameter in normal nerves (Table 4*a, b*) give a sharply unimodal size frequency distribution in both the large fibre and small fibre bundles. In the four laryngeal bundles measured there is a close grouping of fibres around a single peak at 8–10 or 10–12 $\mu$  and the nerves contain no medullated fibres below 4 $\mu$ . The number of small medullated fibres passing from the vagus into the recurrent laryngeal nerve is variable, with a range from about 400 to 600 and these also have a sharply unimodal size-frequency distribution with a mode at about 4 $\mu$ . No fibres larger than 8 $\mu$  have been found in these bundles, and in all instances over 90% of the fibres



Table 4a. *Size-frequency distribution of medullated fibres in the normal laryngeal bundle of the recurrent laryngeal nerve near its origin from the vagus and at its entry into the larynx*

Animal and specimen	Level	No. of fibres in diameter groups of $2\mu$								Total
		0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	
9b	Origin	0	0	5	30	69	105	25	0	234
9c	Larynx	0	0	14	22	64	96	34	11	241
7b	Origin	0	0	14	28	97	115	23	3	280
7c	Larynx	0	0	5	12	55	119	73	5	269
48a	Origin	0	0	20	50	111	57	0	0	238
48b	Larynx	0	0	2	41	133	55	19	0	250
49a	Origin	0	2	20	137	102	49	4	0	314
49b	Larynx	0	0	6	44	108	111	37	0	296

Table 4b. *Size-frequency distribution of medullated fibres in the normal small fibre (sensory) portion of the recurrent laryngeal nerve near its origin from the vagus*

Animal and specimen	No. of fibres in diameter groups of $2\mu$				
	0-2	2-4	4-6	6-8	Total
7b	41	234	180	34	489
48a	16	286	143	24	469
49a	52	417	84	8	561

were smaller than  $6\mu$ . The recurrent laryngeal nerve contains relatively few non-medullated fibres and most of these survive after cutting the cervical vagus trunk caudal to the nodose ganglion (Table 5). Presumably the majority are post-ganglionic sympathetic fibres, having a vasomotor function in the laryngeal muscles.

Table 5. *Counts of non-medullated fibres in the laryngeal bundle of the recurrent laryngeal nerve, comparing the number in normal specimens with the number after chronic section of the cervical vagus caudal to the nodose ganglion*

Normal		Operated	
Animal and specimen	Count	Animal and specimen	Count
(3374 C), 42a	81	(3438 D), 43a	68
(3383 B), 23b	61	(3105 G), 44a	54
(3161 D), 22b	52	(3384 E), 37b	35
(3414 E), 29c	33	(3349 G), 34b	33
(3465 H), 30b	25		
(3376 G), 20c	22		

Interruption of the vagus intracranially or extracranially above the nodose ganglion regularly produces clear-cut changes in the recurrent laryngeal nerve. All the large fibres in the laryngeal bundle degenerate leaving only a few fibres of between 4 and  $6\mu$  in diameter. The number of these fibres remaining in six laryngeal bundles after supranodose (extracranial) vagotomy were 7, 5, 3, 1, 0 and 0 respectively, whilst two nerves after intracranial vagotomy contained 5 and 3 fibres. These remaining fibres were probably not vagal in origin, as a few fibres survived in the laryngeal branch of the recurrent laryngeal nerve after chronic section of the

cervical vagus trunk *below* the nodose ganglion. In five such nerves 6, 5, 2, 0 and 0 medullated fibres were found. All nerve fibres of vagal origin present in the laryngeal branch of the recurrent laryngeal nerve are therefore efferent.

*Thoracic vagus at the upper thoracic level*

Below the origin of the recurrent laryngeal nerve the vagus trunk consists mainly of non-medullated fibres with small and medium calibre medullated fibres scattered throughout the section. Table 6, *a* shows the size-distribution of medullated fibres in five normal nerves at the level mid-way between the aortic arch and the hilum of the lung. The fibres range in a unimodal fashion from 1 to 12  $\mu$  in diameter, with a peak in the 2–4  $\mu$  or 4–6  $\mu$  group. The great majority of these fibres are distributed in the bronchial branches.

Table 6. *Size-frequency distribution of myelinated fibres in the thoracic vagus nerves at a level mid-way between the aortic arch and the hilum of the lung*

(L (left) and R (right) denote the side from which the nerve was taken.)

Animal and specimen		No. of fibres in diameter groups of $2\mu$							Total
		0-2	2-4	4-6	6-8	8-10	10-12	12-14	
(a) Normal									
10 <i>b</i>	R	16	232	361	122	44	5	0	780
11 <i>b</i>	R	28	652	668	265	112	0	0	1725
12 <i>a</i>	R	67	638	584	174	41	7	0	1511
13 <i>a</i>	L	12	504	556	186	17	0	0	1275
14 <i>a</i>	L	52	391	503	176	62	2	0	1186
(b) Supranodose vagotomy									
10 <i>c</i>	L	38	370	427	171	91	18	0	1115
11 <i>c</i>	L	26	426	653	221	143	10	0	1478
12 <i>b</i>	L	18	543	749	156	69	8	0	1543
15 <i>b</i>	L	5	381	458	191	42	0	0	1104
16 <i>a</i>	L	49	343	306	162	45	1	0	906
(c) Intracranial vagotomy									
26 <i>c</i>	L	20	602	485	235	113	9	0	1464
27 <i>c</i>	L	12	407	443	177	97	70	9	1215

Examination of the upper thoracic vagus nerves after chronic supranodose or intracranial vagotomy (Table 6, *b* and *c*) shows that the majority, and possibly all, of the medullated fibres at this level are sensory. These procedures induced no statistically significant change, either in the total number of medullated fibres or in the proportion within each diameter group. Table 6 also indicates that most, if not all, of the afferent medullated fibres in the thoracic vagus nerve have their cell bodies in the nodose ganglion, the jugular ganglion contributing few or none.

*Cardiac branches*

The thoracic cardiac branches of the vagus are variable in their number, size and level of origin. The majority arise at the root of the neck and the lowest filaments emerge from the left recurrent laryngeal nerve or from the vagus at the level of the aortic arch. The cardiac branches are also variable in their histological appearances. Most contain both non-medullated and medullated fibres. The latter range from 1 to 12  $\mu$  in diameter, the majority being at the lower end of the range.

Supranodose or intracranial vagotomy produced variable changes in the fibre composition of the individual cardiac branches. In Weigert stained sections some bundles showed little change, whilst in others all, or nearly all, of the medullated fibres had degenerated (Pl. 2, figs. 8–10). Similar variations were encountered in the non-medullated components; in some branches there was considerable degeneration and in others almost none. This indicates that cardiac afferent and efferent stimuli are carried by both medullated and non-medullated fibres.

#### *Bronchial branches of the vagus*

These are one to three in number and arise at the level of the root of the lung, where they are joined by filaments from the sympathetic trunk. These branches contain both medullated and non-medullated fibres, the former range from 1 to  $12\mu$  in diameter and include most of the large fibres present in the thoracic vagus trunk.

After intracranial or supranodose (extracranial) vagotomy many non-medullated fibres in the bronchial branches degenerated (Pl. 2, figs. 11, 12) but in all instances some of these remained. There was no evidence of a reduction in the number of medullated fibres, either in the individual bronchial branches or in the upper thoracic vagus trunk. It may be concluded, therefore, that the vagal motor innervation of the bronchi in the rabbit is essentially by non-medullated fibres, and that sensory stimuli are carried by medullated fibres with a wide range of diameter and by a few non-medullated fibres.

#### *The abdominal vagus trunk*

##### *(a) Composition of the normal abdominal vagi*

In the rabbit the vagi enter the abdomen as two main nerve trunks lying ventral and dorsal to the oesophagus. Occasionally there are additional smaller twigs, some of which are embedded in the connective tissue surrounding the oesophagus. These nerves contain very few medullated fibres (Pl. 3, fig. 17), there being less than 75 in each of four animals investigated but sections stained with the pyridine-silver method show bundles of closely packed axons (Pl. 3, fig. 13). Estimates were made of the number of axons entering the abdomen in six normal vagus nerves (Table 7, *a*) giving a mean of  $26,178 \pm 1315$ .

##### *(b) Disposition of the fibres from the right and left cervical vagi in the abdominal vagus trunk*

In the rabbit the dorsal and ventral abdominal vagus trunks derived their fibres in approximately equal numbers from the right and left cervical vagi (Table 7, *b* and *c*). At the level of the diaphragm the nerve trunks contain fibres from both vagus nerves, the left contributing mainly to the ventral and the right to the dorsal trunk. Chronic section of one cervical vagus thus results in fibre degeneration in a segment of each trunk at the level of the diaphragm, the demarcation into zones of high and low fibre density being more or less distinct (Pl. 3, fig. 15). As the nerves descend into the abdomen these zones become less obvious as more intimate mixing of the fibres occurs.



Table 7. *Estimate of the total number of axons entering the abdomen in the vagus nerves in normal and operated rabbits*

Procedure	Animal number	Estimate
(a) Normal	28	22,896
	29	24,074
	22	30,654
	30	29,297
	20	26,571
	31	23,574
(b) Left cervical (infranodose) vagotomy	34	13,138
	35	16,096
(c) Right cervical (infranodose) vagotomy	36	15,197
	21	14,356
	37	14,243
	38	14,353
(d) Left cervical infranodose vagotomy + right upper thoracic vagotomy	39	1,372
	40	1,721
	41	569
(e) Left supranodose vagotomy + right upper thoracic vagotomy	32	15,848
	24	10,731
(f) Left supranodose vagotomy	25	27,023
	33	28,632
(g) Left intracranial vagotomy	26	26,116
	27	27,488

*(c) Adventitial fibres in abdominal vagi*

In three rabbits subjected to chronic left-sided cervical vagotomy and right-sided upper thoracic vagotomy the abdominal vagus nerves were examined in order to determine the number of fibres derived from sources other than the cervical vagi. Counts of 1721, 1372 and 569 fibres were obtained (Table 7, *d*) and in silver-stained material (Pl. 3, fig. 14) these were found sparsely distributed throughout the section. These fibres thus account for about 5% of the total fibre content of the normal abdominal vagus nerves. Many of these fibres probably reach the vagus through communications with the sympathetic chain at variable levels in the thorax (Molhant, 1913). Others may be visceral afferent fibres leaving the thoracic vagus trunks to join the intercostal nerves close to their exit from the intervertebral foramina and entering the spinal cord by the dorsal roots. Fibres of this latter type have been described in the cat by Harper, McSwiney & Suffolk (1935).

*(d) Sensory fibres in the abdominal vagus nerves*

In order to estimate the number of sensory fibres entering the abdomen in the vagus nerves, chronic right upper thoracic vagotomy and left supranodose vagotomy was performed in two rabbits.

Table 7, *e* shows the total number of fibres entering the abdomen in vagus nerves following the procedure outlined above (Pl. 3, fig. 16). The data are not adequate to show accurately the relative proportions of sensory and motor fibres but they suggest that the latter are very few, probably accounting for less than 10% of the total. This conclusion is supported by fibre counts made on the abdominal nerves of rabbits, subjected to unilateral (left) supranodose or intracranial vagotomy (Table 7, *f* and *g*). Indeed, these latter counts are within the normal range, but microscopic

examination of the silver stained specimens show small areas of low-fibre density in which efferent fibres have undergone degeneration.

Of the few medullated fibres present in the abdominal vagi, some were sensory and some were adventitial in origin. This was shown by the fact that 68 and 54 remained after upper thoracic vagotomy and left supranodose vagotomy, and 85 and 24 remained after upper thoracic vagotomy and left cervical vagotomy.

#### DISCUSSION

This quantitative study has shown that the vagus nerve of the rabbit is composed predominantly of afferent fibres. We have also shown that the great majority of the nerve fibres in the vagus trunk at all levels are non-medullated and that the proportion of these fibres increases rapidly as the nerve is traced into the abdomen where it contains very few medullated fibres.

#### *The function of the cells in the nodose and jugular ganglia*

A number of workers have suggested that a proportion of the motor fibres in the mammalian vagus nerve have their parent cells in the ganglion nodosum. The evidence is based on experiments in which the vagus was divided intracranially or extracranially above the ganglion nodosum and time allowed for fibres deprived of their cells to degenerate. In the dog, Morgan & Goland (1932) reported variable effects on the blood pressure and heart rate when the cervical vagus-sympathetic trunk thus treated was stimulated electrically. The effects they obtained may, however, have been due to stimulation of descending fibres in the cervical sympathetic trunk (Foley, 1945; Butson, 1950; Daly & Hebb, 1952). In the cat, Heinbecker & O'Leary (1933 *a, b*), stimulated the cervical vagus, the fibres of which had been allowed to degenerate for 10–20 days after intracranial or supranodose (extracranial) vagotomy, and claimed that this produced broncho-constriction and either inhibition or excitation of peristalsis in the duodenum. On the other hand, Daly & Evans (1953), using similar operative procedures in the cat, could find no such residual broncho-constrictor effect. Heinbecker & O'Leary found no residual cardiac effects after supranodose vagotomy in the cat, and this was confirmed by McSwiney & Spurrell (1933), Richardson & Hinsey (1933), and Daly & Evans (1953). The present study indicates that there are no motor fibres with their cell bodies in the nodose ganglion. Molhant (1913) found, in the rabbit, that the cells near the upper pole of the nodose ganglion provide sensory fibres which pass into the superior laryngeal and cardiac depressor nerves, the central part to the recurrent laryngeal nerve whilst the thoracic and abdominal viscera are innervated with sensory fibres having their cell bodies mainly towards the lower pole.

As regards the jugular ganglion, Molhant (1913) believed that a number of sensory fibres in the laryngeal bundle of the recurrent laryngeal nerve of the rabbit have their cell bodies in the ganglion. His findings were almost certainly due to regeneration of motor fibres as there was a lapse of 7 months between operation and biopsy. Our results indicate that all the large fibres destined for the intrinsic laryngeal muscles degenerate after extracranial supranodose vagotomy or after intracranial vagotomy. In the cat, Heinbecker & O'Leary (1933*b*) and Dubois & Foley (1936)

found that the majority, if not all, of the sensory fibres of the recurrent laryngeal nerve, i.e. the small fibres, have their cell bodies in the nodose ganglion. The jugular ganglion cells in the cat distribute their fibres mainly into the auricular branch, which Molhant had not investigated (Foley & Dubois, 1937).

#### *Afferent fibres from the abdomen in the vagus nerves*

The extent of the preponderance of sensory fibres in the case of the abdominal vagus is surprising, as the evidence so far obtained from physiological investigations suggests that the vagus plays a relatively minor role in the transmission of afferent impulses from the abdominal viscera. It is generally considered that the splanchnic nerves constitute the chief sensory pathway from these viscera, and clinical evidence supports the view that afferent impulses carried in the vagus do not enter consciousness. The difficulty in interpreting the physiological findings may be appreciated from the following brief review.

Müller (1911) asserted that there is no evidence that afferent impulses are carried in the abdominal vagus, and Cannon (1933) stimulated the vagus with buried electrodes, in unanaesthetized cats, without producing distress or discomfort. Lennander (1906) and Foerster (1927) both considered that the abdominal vagus contains no pain fibres. Grimson, Hesser & Kitchin (1947) stimulated the abdominal vagus nerves in patients under spinal anaesthesia and reported that no sensation was aroused. Abdominal vagotomy used in the treatment of peptic ulceration often produced relief of the ulcer pain, but this is believed to result from reduction in gastric acidity and in peristaltic activity rather than from interruption of afferent fibres (Grimson *et al.*). Similarly, Grossman & Stein (1948) found that although vagotomy produced relief of pain associated with hunger contractions in the stomach, this relief was caused by stopping the excessive contractions rather than by cutting the sensory pathway.

On the other hand, a number of workers have found that stimulation of the central cut end of the abdominal vagus nerves produced reflex effects in the animal. Brodie & Russell (1900) stimulated the vagus in dogs and cats and recorded a small and variable slowing of the heart rate and fall in blood pressure after a preliminary slight rise in pressure. Neuman (1914) found that stimulation in both the cat and rabbit caused a rise in blood pressure accompanied in the cat by reflex body movement, vomiting and increase in respiration. In the rabbit these latter effects were usually absent. The course of afferent fibres running in the abdominal vagus of the cat was investigated by Harper *et al.* (1935) who used dilation of the pupil as an index of afferent nerve activity. They subdivided these visceral afferents into two groups according to the pathway by which they reach the central nervous system. One group, having their cell bodies in the nodose ganglion, pass in the vagus to the medulla. They claimed that the other group leave the vagus in the thorax and run along the intercostal arteries to join the intercostal nerves, passing into the spinal cord in the dorsal roots of the second to the eighth thoracic nerves inclusive.

It is evident from the foregoing that much still remains to be learned regarding the functions of the afferent fibres from the abdominal viscera contained in the vagus nerve.



*Efferent fibres to the abdomen in the vagus nerves*

In view of the present finding that less than 10% (about 3000) of the abdominal vagal fibres are efferent, it is difficult to understand how they could innervate the extensive abdominal territory over which the vagus is distributed.

It is presumed that the preganglionic fibres of the vagus make synaptic connexions with the ganglion cells of the enteric plexuses, although there is little histological information in the nature of the endings. From the work of Irwin (1931) and Matsuo (1934) it may be estimated that there are several million nerve cells in Auerbach's plexus in the gastro-intestinal tract. No figures are available in the case of Meissner's plexus or for other abdominal organs innervated by the vagus. It seems improbable that the relatively few motor fibres present in the abdominal vagus establish synaptic connexions with all the enteric ganglion cells, as this would involve a ratio of preganglionic to postganglionic neurones far higher than the ratios found elsewhere in the autonomic nervous system. Langley (1922) called attention to this discrepancy and suggested that the vagus fibres make synapse with a small proportion of the cells in the enteric plexuses. He postulated that these special 'mother cells', each connected to a vagal fibre, distributed the impulses to a large number of enteric nerve cells. There is no histological evidence for the presence of such intermediate neurones.

*The recurrent laryngeal nerve*

The branch of the recurrent laryngeal nerve which enters the larynx to innervate the intrinsic laryngeal muscles has a fibre calibre distribution which is characteristic of various muscle nerves in the head and neck. Fernand & Young (1951) studied the diameters of fibres in nerves supplying a wide variety of muscles in the rabbit and distinguished two types of nerves. The 'unimodal' muscle nerves have a single sharp peak at about  $10\mu$ , with few fibres of smaller or larger diameter. This type of nerve was found to innervate the infrahyoid muscles, the diaphragm and the superficial muscles of the face. The 'bimodal' muscle nerves, on the other hand, have two distinct peaks representing large and small fibres, with few of intermediate size (about  $10\mu$ ). A considerable proportion of the fibres are below  $6\mu$  and above  $14\mu$  in diameter. These 'bimodal' nerves supply most of the limb muscles and also the extrinsic ocular muscles.

Fernand & Young suggested that those nerves with a 'unimodal' distribution were connected to muscles which had little or no proprioceptor supply. The absence in these 'unimodal' nerves of the large and small medullated fibres which are present in 'bimodal' muscle nerves (Eccles & Sherrington, 1930; Lloyd & Chang, 1948; Rexed & Therman, 1948) would thus be explained. The 'unimodal' nerves may therefore be composed almost entirely of one type of fibre—extrafusal efferent.

Molhant (1912) reported that the intrinsic laryngeal muscles of the rabbit are innervated entirely by the recurrent laryngeal nerve, the superior laryngeal nerve making no contribution. The finding in the present investigation of complete absence of afferent medullated fibres from the branch of the recurrent laryngeal nerve innervating the laryngeal muscles thus supports the suggestion of Fernand & Young. Furthermore, those who have searched for muscle spindles in the intrinsic

laryngeal muscles report that none are present (Cipollone, 1897; Baum, 1900). We feel that further work might profitably be done on a comparative study of the fibre composition of muscle nerves correlating the results with quantitative studies on spindles and other afferent endings in the muscles.

#### SUMMARY

1. After chronic section of the vagus nerve of the rabbit intracranially or extracranially above the nodose ganglion, stimulation of the caudal end of the nerve produces no effect on the heart, larynx, oesophagus or stomach. It is concluded, therefore, that none of the motor fibres in the vagus supplying these structures have their cell bodies in the nodose or jugular ganglia.

2. The cervical vagus contains about 23,000 fibres, of which only about 2900 or 13% are medullated. Between 20 and 25% of the total are efferent. Nearly all the large (above  $10\mu$ ) and 40–50% of the small (below  $4\mu$ ) medullated fibres are efferent. On the other hand, probably all the medium-size medullated fibres ( $4$ – $10\mu$ ) are afferent.

3. The branch of the recurrent laryngeal nerve innervating the laryngeal muscles contains large medullated fibres with a sharply unimodal size-frequency distribution. All these fibres degenerate after cutting the vagus above the ganglia and are, therefore, efferent. The probability that the laryngeal muscles of the rabbit are devoid of proprioceptive innervation is discussed.

4. The thoracic cardiac branches of the vagus are very variable in composition. They contain afferent and efferent fibres of both the medullated and non-medullated varieties.

5. The motor functions of the vagus to the bronchial musculature are served by non-medullated fibres. On the other hand, pulmonary afferent stimuli are conveyed by medullated fibres with a range of diameter between 1 and  $12\mu$  and by a few non-medullated fibres.

6. The vagus nerves as they enter the abdomen contain about 26,000 non-medullated fibres, of which probably less than 10% are motor. There are very few medullated (less than 75) present at this level. The abdominal vagus trunks derive their fibres in approximately equal numbers from the right and left cervical vagi.

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## EXPLANATION OF PLATES

With the exception of Pl. 1, fig. 5, all figures are from transverse sections of the vagus.

## PLATE 1

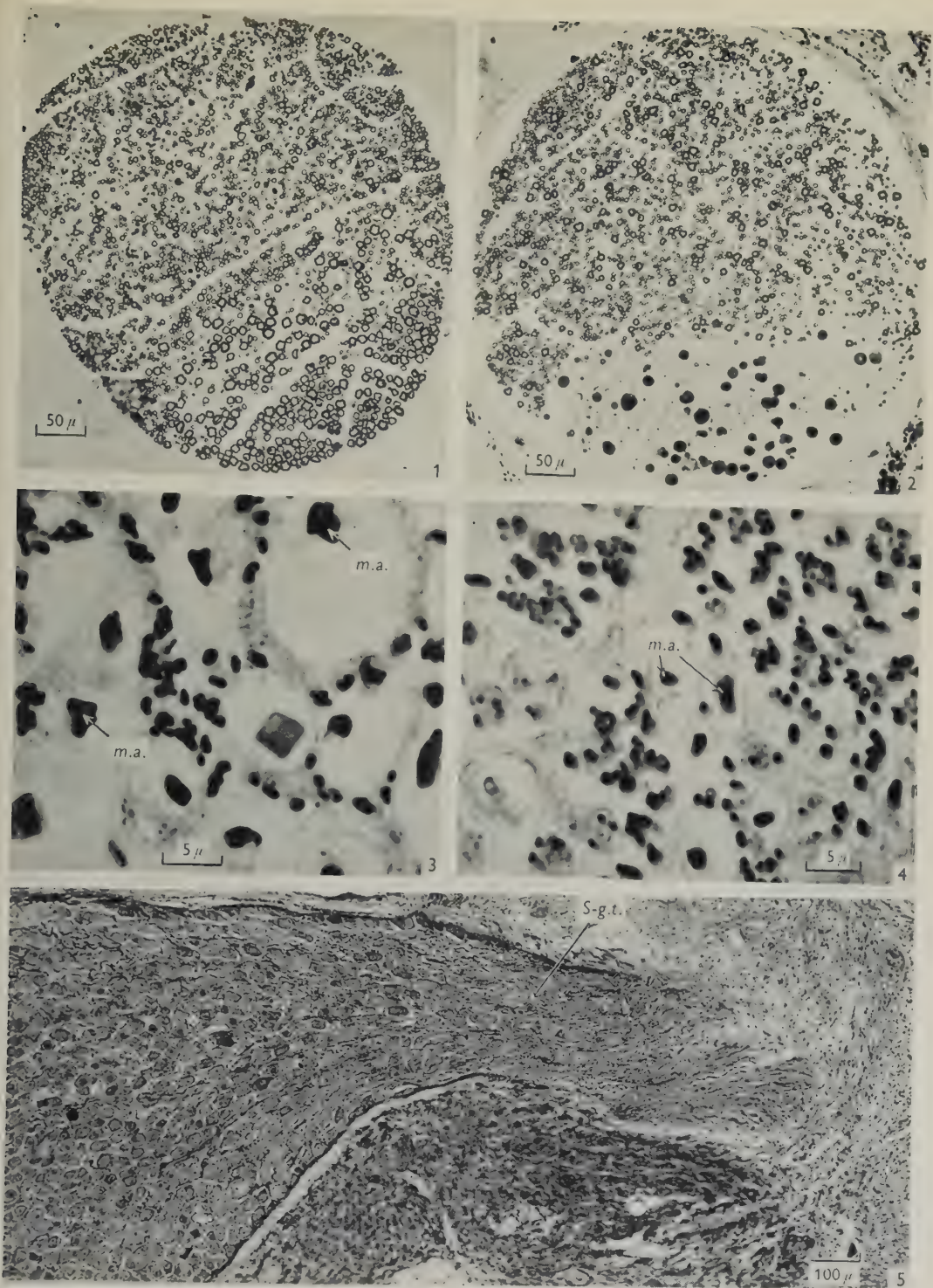
- Fig. 1. Normal rabbit vagus at the lower cervical level, showing the distribution of medullated fibres. The large fibres occupying the lower third of the section represent the motor innervation of the laryngeal muscles. Weigert stain.
- Fig. 2. Lower cervical vagus 21 days after cutting the vagus extracranially between the nodose ganglion and the base of the skull. The large medullated fibres which are motor to the laryngeal muscles have degenerated. Some of the myelin droplets have not been absorbed. The remaining part of the section shows a density of medullated fibres approximately equal to normal. Weigert stain.
- Fig. 3. Portion of vagus at the lower cervical level, showing groups of non-medullated axons amongst the medullated fibres. The axons of the latter (*m.a.*) have undergone considerable shrinkage. The segment of nerves shown is in the region containing the large laryngeal medullated fibres. Pyridine-silver stain.
- Fig. 4. Portion of lower cervical vagus 21 days after supranodose (extracranial) section of the nerves. The segment selected is in the region containing small and medium calibre medullated fibres (upper two-thirds of Pl. 1, fig. 2) and shows typically a fibre density approximately equal to normal. Pyridine-silver stain.
- Fig. 5. Longitudinal section of the upper pole of the nodose ganglion and the neuroma which has developed during the 21 days succeeding supranodose (extracranial) section of the nerve. Examination of the nerve cells in the supraganglionic nerve trunk (*S-g.t.*) under high magnification shows that the majority are apparently undamaged. Bodian stain, after fixation in Bouin's fluid.

## PLATE 2

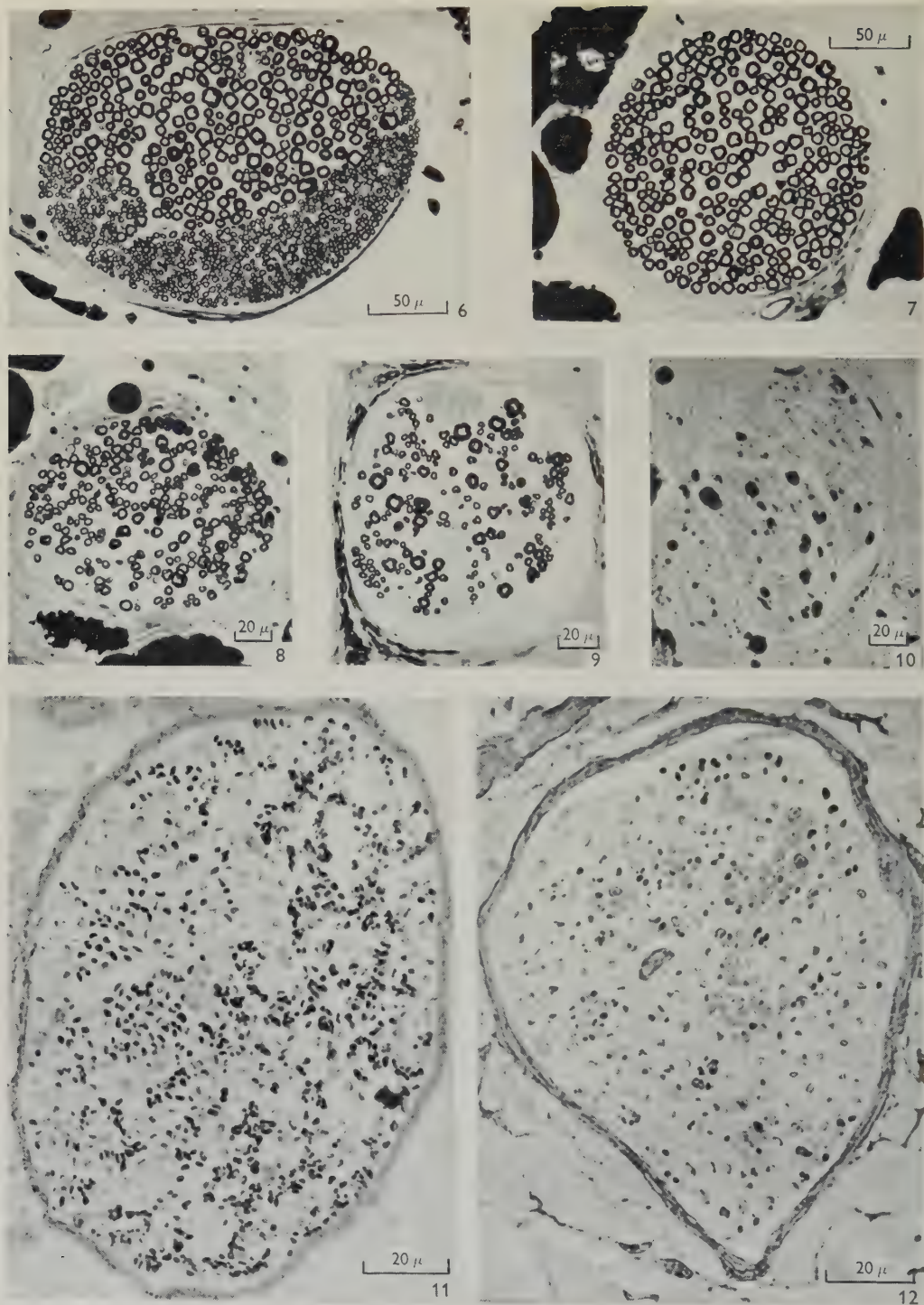
- Fig. 6. Normal recurrent laryngeal nerve close to its origin from the vagus. The large and small medullated fibre groups occupy separate segments of the nerve. Weigert stain.
- Fig. 7. Normal recurrent laryngeal nerve at its entry into the larynx. Only the large medullated fibres pass to larynx, the small medullated group having been distributed in branches to the heart, trachea and oesophagus. Weigert stain.
- Fig. 8. Normal cardiac branch of the vagus showing small and medium calibre medullated fibres. Weigert stain.
- Fig. 9. Cardiac branch after intracranial vagotomy showing that the majority of the medullated fibres have survived in this instance. Weigert stain.
- Fig. 10. Cardiac branch after intracranial vagotomy showing degeneration of all the medullated fibres. Weigert stain.
- Fig. 11. Normal bronchial branch of the vagus containing darkly stained non-medullated axons and lightly stained medullated fibres. Pyridine-silver stain.
- Fig. 12. Bronchial branch after intracranial vagotomy. The majority of the non-medullated axons have degenerated, but probably all of the medullated fibres are intact. Pyridine-silver stain.

## PLATE 3

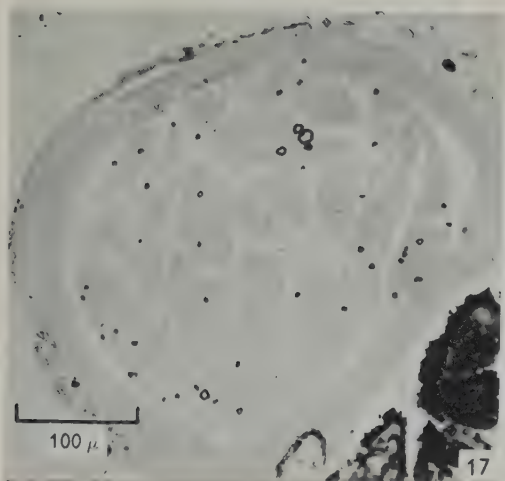
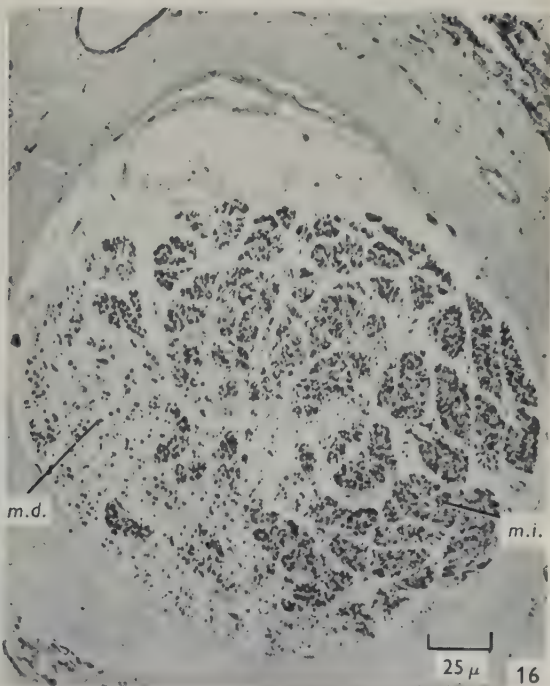
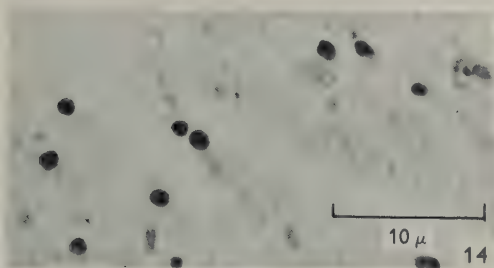
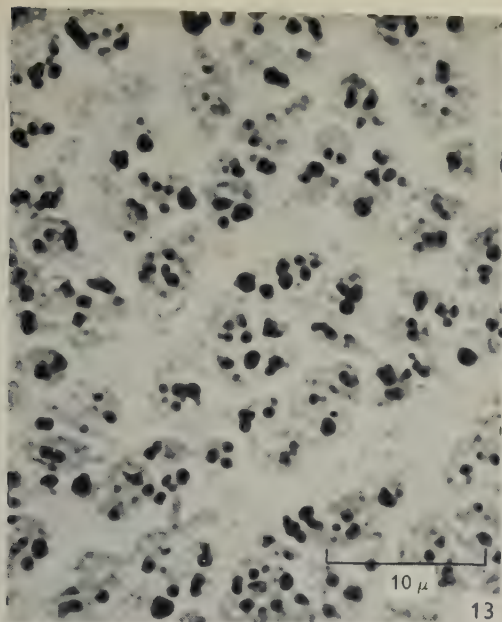
- Fig. 13. Segment of normal abdominal vagus with its high and uniform population of non-medullated axons. Pyridine-silver stain.
- Fig. 14. Portion of abdominal vagus trunk after bilateral vagotomy. The right vagus was cut in the upper thorax and the left vagus, cut at the level of the thyroid cartilage. Nearly all the non-medullated fibres have degenerated. Pyridine-silver stain.













- Fig. 15. Low-power view of the posterior abdominal vagus trunk after chronic section of the right cervical vagus below the nodose ganglion. Discrete areas of mainly intact (*m.i.*) and mainly degenerated (*m.d.*) areas are seen. Pyridine-silver stain.
- Fig. 16. Low-power view of the posterior abdominal vagus after right-sided upper thoracic vagotomy and left-sided supranodose vagotomy. Areas of mainly intact (*m.i.*) and mainly degenerated (*m.d.*) fibres can be distinguished. Pyridine-silver stain.
- Fig. 17. Normal anterior abdominal vagus trunk containing a very few small (below  $6\mu$ ) medullated fibres. Weigert stain.



# THE DEVELOPMENT AND GROWTH OF THE PLACENTOMES IN THE FALLOW DEER (*DAMA DAMA* L.)

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## INTRODUCTION

Previous accounts of the placentae of the Cervidae (Turner, 1878*a, b*, 1879; Strahl, 1906, 1911; Kolster, 1909; Andresen, 1922, 1927; Harrison & Hamilton, 1952) have generally been handicapped by the lack of a series of specimens representative of all the stages of pregnancy. The authors quoted above have had to rely, for their observations and comparisons, on only a few specimens obtained at random during the period of pregnancy; for a number of species there is not even a description of the non-pregnant uterus. There is no detailed description of the placenta and membranes of *Dama dama*, but it is hoped that such a description will appear shortly (Hamilton & Harrison, unpublished). The following observations will be limited to those made on the growth, changes in form and gross structure of the maternal (caruncular) and foetal (cotyledonary) elements of the placentome from the time of the development of the cotyledonary villi until the foetus has reached a c.r. length of 40 cm.

As far as is known the Fallow Deer no longer exists in a truly wild state in Great Britain, but is only found in herds preserved in certain parks and Zoological Gardens; some authorities, however, are of the opinion that the fallow deer in Epping Forest can be considered to be living in a wild state. Mating in parks in southern England occurs throughout October, but may be extended into November. Twins are rare, one instance only being found in the present series; other instances have been reported to us personally by the late Duke of Bedford. Parturition occurs in late May and continues into July; the fawns can run rapidly 7 hr. after birth.

## MATERIALS AND METHODS

The material available consists of over 250 specimens of non-pregnant and pregnant animals obtained from Petworth Park, Richmond Park and Knole Park, from 1951 to 1953. The animals were shot and the reproductive tracts and ovaries removed at once, or as quickly as possible after death. The specimens were dealt with in a number of ways; many were fixed entire in 10 % formalin, Bouin's or Rossman's fixative; forty were opened before fixation and the allantoic and amniotic fluids removed for analysis (Walker, 1954); the remainder were opened and parts of the membranes, placentomes and uterus were fixed in chilled acetone, Rossman's fluid, 80 % alcohol, basic lead nitrate or Zenker formol. Many of the specimens were injected with coloured gelatin, Marco resin or Latex through the foetal and maternal circulation. The ovaries were removed from every specimen and portions fixed in the fluids mentioned above. The chorionic sac was removed entire from a number of

the more recently pregnant specimens and the cotyledons were excised and fixed on pieces of cork. The number of villi in each cotyledon was estimated by counting them on a photograph taken of the cotyledon under water. After the formation of the placentome it was found to be quite easy in the early stages to separate the cotyledon from the caruncle. The villi could then be counted independently by plucking them one by one from the detached cotyledon. The caruncular crypts were counted in two ways. The surface area of the crypt-bearing portion of the caruncle was estimated as accurately as possible. The number of crypts was then counted in a series of areas 4 mm. square selected at random over the crypt-bearing surface. The mean of the counts was determined, and, knowing the surface area, the total number of crypts could be calculated. It was found by experiment on those caruncles in which the total number of crypts was known by the method described below that if the mean number of crypts in five areas 4 mm. square was counted, then the estimated number of crypts fell within a range  $\pm 5\%$  of the true number.

The second method was more accurate and was used to check the first. The caruncle was embedded in 15% gelatin, and after cooling the gelatin was cut off with a knife flush with the surface of the caruncle. The crypts could then be counted individually; the position of each was marked off with a fine pen under a dissecting microscope so that it would not be counted twice.

The separation of the villi from the caruncle at later stages was more difficult, and it was found that the number of villi could be more quickly assessed by carefully stripping the chorion away from the caruncle and leaving the remnants of the bases of the villi intact on the cotyledon. The cotyledon could then be stretched out on cork and the number of villi counted under a low-power microscope. The number of crypts in the caruncles of later stages was estimated by the methods described above, and although the results are not as accurate as in the earlier stages it has been shown by comparison of the totals derived from the application to a few well-developed caruncles of both methods described above that the counts at later stages are probably accurate to within  $\pm 10\%$ .

#### THE NON-PREGNANT UTERUS

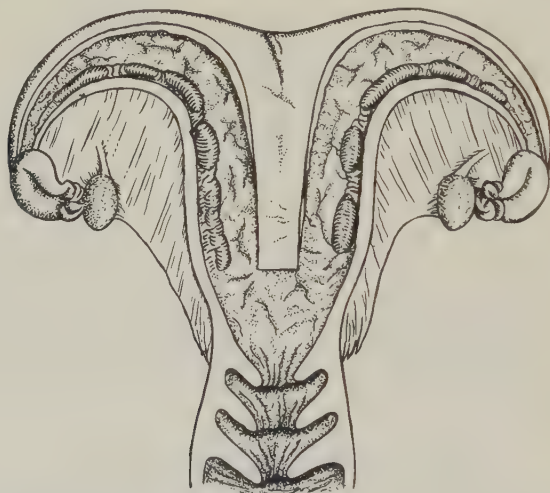
Eleven specimens are available for a description of the appearance of the uterus before pregnancy occurs (Table 1). The macroscopic appearances of the non-pregnant uterus are essentially similar to those already described for another species (Harrison & Hamilton, 1952), except in respect of the number and shape of the caruncles.

Three animals are about 8 months old, and in these specimens the uterine horns are 3.5 cm. in length in their unfused portions, and 6-7 mm. in diameter nearest the point where the two horns become fused. The fused portion of the uterus is 1 cm. in length and 1 cm. in diameter; the common chamber is 5 mm. long. The distance from the internal os to the lowest of three large vaginal folds is 3.0 cm., and the cervix is 1 cm. in diameter. The internal appearances of the uterine horn show an almost smooth mucosa with the small, sessile or leaf-like caruncles projecting into the lumen. The surface of the caruncles is quite smooth and shiny. The common chamber of the uterus shows numerous parallel longitudinal folds about 0.5 mm. broad.

The uteri obtained from does FD/29, FDE/12 and FDP/13, which are about 18 months old, are all larger than the three described above. The uterine horns are 6 cm. in length and 1.2 cm. in diameter; the fused horns are 2–3 cm. in length and 1–2 cm. in diameter.

Table 1. *Non-pregnant does*

Animal no.	Date killed	Remarks
FDE/48	27. i. 53	8 months old, few small follicles in both ovaries
FDE/77	17. ii. 53	8 months old, few small follicles in both ovaries
FDE/87	23. ii. 53	8 months old, few small follicles in both ovaries
FD/29	16. i. 52	18 months old, recently ovulated
FDE/12	2. xii. 52	18 months old, recently ovulated
FDP/13	1. xii. 53	18 months old, recently ovulated
FDE/18	2. xii. 52	Adult, macerated foetus impacted in vagina, recently ovulated
FDE/46	20. i. 53	Adult, recently ovulated. Peritoneal adhesions and evidence of having been previously wounded
FD/14	18. xii. 51	Adult, corpus luteum present, tumour in broad ligament
FDE/70	10. ii. 53	30 months old, not ovulated, no corpus albicans
FDE/34	14. i. 53	Adult, corpus albicans, no signs of recent ovulation



Text-fig. 1. A drawing of the inner aspect of the non-pregnant uterus of *Dama dama*, showing the caruncles arranged in a row on the mesometrial border.

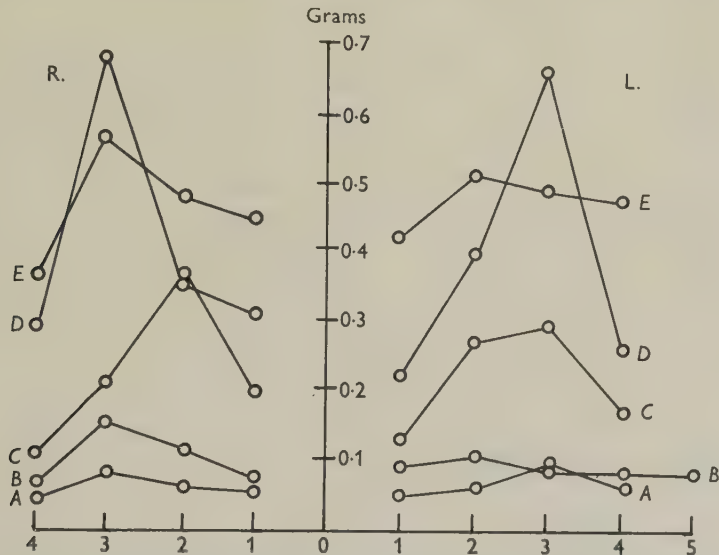
The remaining six non-pregnant uteri are known to be from older does, and the uteri are correspondingly larger. It appears that they are all mature animals which have not been impregnated for one reason or another (Table 1). One of these (FDE/18) is of interest in that an almost completely macerated foetus was found impacted in the vagina. The doe was killed at a time when the average length of the embryo in the other pregnant specimens was 20 mm. From examination of the lengths of the long bones it is estimated that the foetus of this specimen was about 350 mm. in c.r. length. It is therefore suggested that the doe FDE/18 was one which was caused to abort in April 1952, and that the foetus had remained impacted within the vagina until the date on which the doe was finally killed.

The uteri from these specimens are appreciably larger than those already described. The uterine horns are 8–9 cm. in length, 1.4–1.6 cm. in diameter for the



major part of their extent, but taper rapidly at the tubal end to 3–5 mm. in diameter. The fused horns are 6 cm. in length and 1·7 cm. across; the common uterine chamber is 1·5 cm. in length and 1·8 cm. in diameter. The distance from the internal os to the lowest vaginal fold is 3 cm.

The caruncles are arranged in a row along the mesometrial aspect of the uterine lumen (Text-fig. 1). In the young specimens they are separated by an interval which is occupied by folded mucosa. The caruncles from a representative series of the non-pregnant does and from FDE/11, which contained a chorionic sac, were carefully removed and weighed (Text-fig. 2). It will be seen that the commonest number of caruncles in each horn is four, that they weigh between 0·035 and 0·69 g., and that the largest and heaviest caruncles are always situated in the



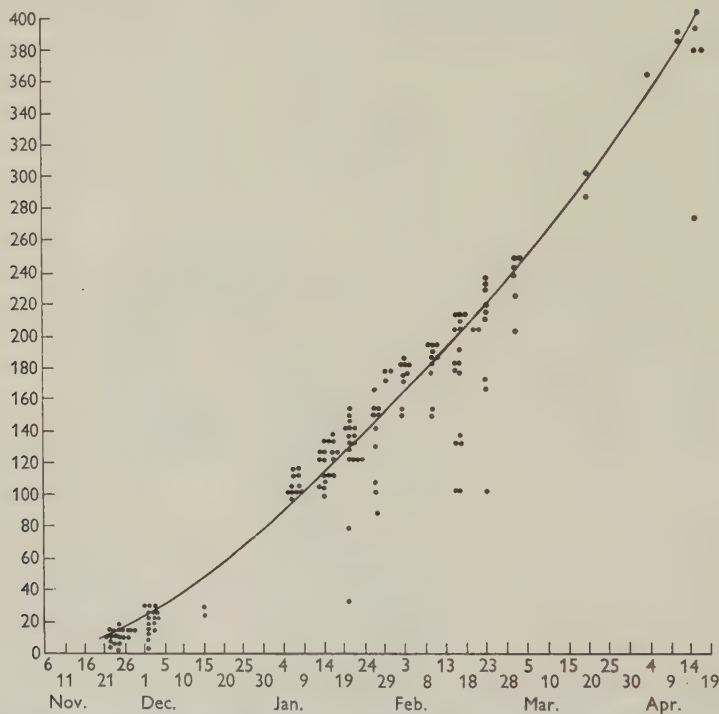
Text-fig. 2. Table to show the weights of individual caruncles removed from the uteri of four non-pregnant does: (A) FDE/77, (C) FDE/70, (D) FDE/18, (E) FDE/46, and one, (B) FDE/11, which contained an early chorionic sac. The central vertical line represents the position of the cervix; those caruncles above the point 1 are nearest the cervix, those above points 4 and 5 are nearest the tubo-uterine junction.

centre of each horn, even in the youngest stages. In the late foetus, also, the central caruncles in each horn appear to be the largest. In the older specimens the caruncles are no longer sessile or leaf-like, but have become elongated ovoids, raised from the uterus by a short, broad pedicle (Text-fig. 1).

These observations suggest that the fallow deer is monoestrous, as is the Roe Deer (Heape, 1901), and that it reaches puberty 15–18 months after birth. This statement is confirmed by records compiled from the system of notching the ears of the newborn fawns adopted at Petworth Park. Not all the does are impregnated during any one breeding season, either because they are too young, or because there is some abnormal condition of the tract, or due to failure of the ovary to produce a mature follicle which is capable of ovulating.

## THE PREGNANT UTERUS

The majority of the pregnant animals were obtained between the end of November and the beginning of April, which covers the period of formation of the placentomes and the greater part of the history of their development. Text-fig. 3 shows the length of the embryos recovered during 1952-3 from Petworth Park, Sussex, plotted against the time of the year. The graph indicates that there is little evidence for delayed implantation. It suggests that there may well be occasional late matings at the end of November.



Text-fig. 3. A graph showing the length in mm. of the embryos recovered from 160 does of *Dama dama*, plotted on the day of the year they were killed at Petworth Park, Sussex, between 21 November 1952 and 14 April 1953. A doe pregnant with twin foetuses was shot on 3 February 1953.

*The chorionic sac*

The youngest chorionic sac recovered (FDE/11) has already acquired its definitive U-shape, with the tips of the U situated at the tubal end of each uterine horn, and the constricted portion between the two expanded arms lying opposite the cervix, and extending round the septum separating the two horns. Twenty-two other specimens (Table 2) were recovered in which the embryo varied in length from 1 mm. (FDE/17) to 27 mm. (FD/6). The following description is based on an examination of all these specimens, but five, FDE/4, FD/15, FDE/10, FDE/5 and FDE/13, were selected for detailed examination.

The arm of the sac in which the foetus is lying is considerably wider and longer than the other. But apart from this there is relatively little difference between the

two arms of the sac in external appearances. The foetus generally lies nearer the cervix than the mid-point of one arm of the sac. The chorion forms a transparent, highly elastic sac which gradually tapers towards its extremities. It contains, for the most part, the allantois filled with allantoic-fluid, which pushes the chorion into relatively close apposition to the uterine wall.

In certain areas, from about the 12 mm. c.r. stage onwards, are the discrete cotyledonary 'plaques', which lie opposite, and from the time of their first appearance, are closely related to, the maternal caruncles. Rising from these plaques are the primitive villous processes, which are destined later to fit into the maternal crypts.

Distributed over the surface of the intercotyledonary membranous part of the chorionic sac are small, whitish, folded elevations—the areolae—which appear to lie opposite the mouths of the uterine glands. No extracotyledonary villi were seen.

The umbilical vessels in the fallow deer consist in two arteries and two veins which lie at first along the lesser curvature of the chorion. One pair of vessels usually supplies and drains each horn of the sac. Branches of these vessels supply the cotyledons. These latter branches pass at random over the foetal side of the chorion, and form no definite topographical pattern on the plaque. The intercotyledonary areas are well supplied, both by separate branches from the umbilical vessels, and also by the terminal parts of the branches supplying the cotyledons. The vessels supplying the chorion break up to form a dense polygonal capillary network, particularly well-marked in the region of the plaques. The veins of the chorion appear to run independently of the arteries.

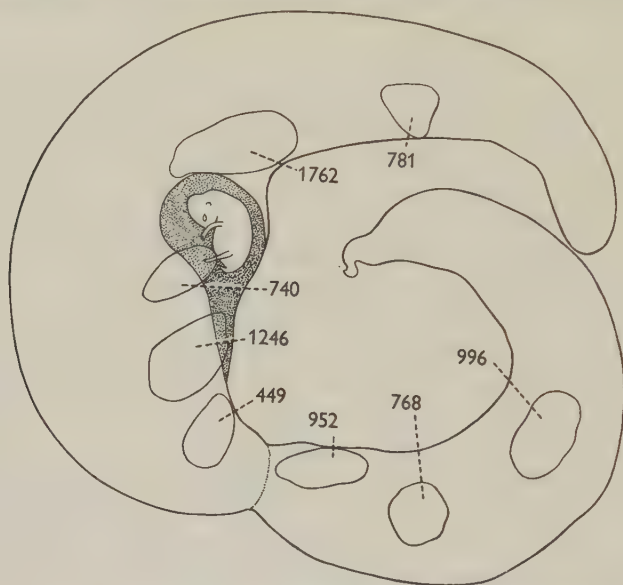
The cotyledonary plaques tend to lie either immediately across the corresponding umbilical vessels or else to the lateral side of them. The branches of the umbilical vessels appear to radiate out from the plaques, leaving the edge of the cotyledon at a large angle (Pl. 1, fig. 1). But within the plaque itself the vessels pass indiscriminately, branching and anastomosing at random.

The plaques all lie on the mesometrial side of the chorion. The usual number of cotyledons in *Dama* is four in each horn (6–12 total). They tend generally to be larger in the pregnant horn, and also tend to be larger if they lie near the foetus, but this is by no means constant. Generally they are smaller near the tips of the chorion; the long axis of each plaque is not always that of the chorion, and frequently lies at an angle to it. The developing cotyledons can be easily excised and pinned out on a sheet of cork. The chorion is markedly elastic, even after fixation, and the degree of stretching prevents any estimate of the size of the cotyledonary plaques relative to the surface area of the caruncle.

Counts have been made of the number of villi in the cotyledons. In FDE/15 (22 mm. c.r. stage) there were eight cotyledons arranged as shown in Text-fig. 4. The larger the plaque, the more villi it contains, but the concentration of villi per unit area on any cotyledon varies in different specimens, and also from plaque to plaque on the same chorionic sac. Thus it was found that in one chorionic sac, two cotyledons having areas of 0.75 and 1.75 cm.<sup>2</sup> approximately, had total villi counts of 726 and 787 respectively. There are not always fewer villi in the cotyledons nearer the tip of the chorionic sac (Text-fig. 5). Similarly, those plaques nearest the foetus do not contain a consistently different number of villi from those situated



elsewhere, as is well shown by FDE/13 and FDE/15 (Text-figs. 4, 5). At the early stages there appears to be no true relation between the position of the plaque and the number of villi that it contains (Text-fig. 5). There is little tendency for those plaques lying at, or near, the mid-point of a limb of the sac to contain more villi than those adjacent to them, thus there is no relation at the early stages between the number of villi in a plaque and the size of the caruncle. Neither is there any significant relation between the size of the villi and the size or position of the plaque at any particular stage. In one specimen (FDE/15) a large cotyledon had villi just



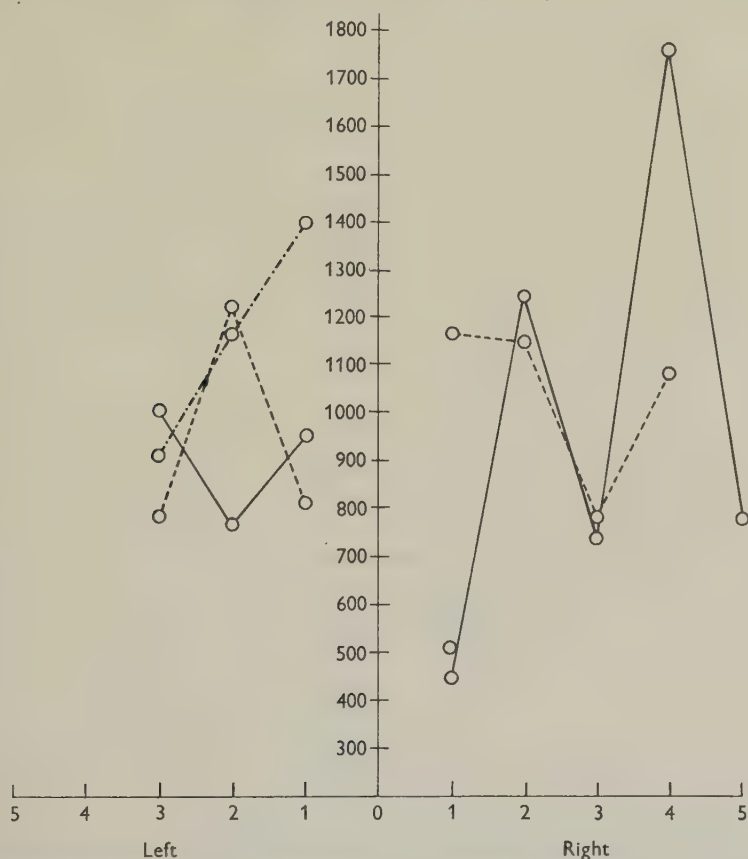
Text-fig. 4. Tracing from a photograph of the intact chorionic sac of FDE/15 to show the relative sizes and distribution of the cotyledonary plaques. The figures indicate the number of villi on each plaque.  $\times \frac{1}{2}$ .

visible to the naked eye, while another, much smaller plaque had villi some 2–3 mm. long. This latter plaque was situated very close to the foetus, whereas the former was at the apex of the opposite horn. Within a particular cotyledon, however, there is a definite relationship between the position of the villus and its size. Those villi nearer the edge of the plaque are always much smaller, and are also farther apart, than those in the centre (Pl. 1, fig. 2).

The villi are arranged in relatively long rows, which lie approximately transverse to the long axis of the chorion, but, as already stated, not necessarily to the long axis of the future placentome. Some of the rows cross the whole width of the plaque. They are not all parallel to each other, but tend to be arranged in interlocking groups of parallel rows. Occasional lines branch or join each other. The rows in the centre of the plaque tend to be straight, whereas those at the ends describe a curve with its concavity towards the plaque. The villous lines are approximately equidistant from each other, as are the individual villi in the row (except at the edges). The rows bear no relation to the vessels of the cotyledon. It must be emphasized that these observations have been made on the intact chorionic sac, removed from

the uterus without any fluids escaping, and subsequently mounted in water. If the fluids escape the chorionic sac collapses, and, owing to the elasticity of the chorion, the linear pattern is much distorted.

This linear arrangement of the villi persists to a varying degree for the first 2 months of pregnancy, but as the foetus gets older there is a tendency for the lines to be replaced by a polygonal arrangement of the villi. Eventually, about the 150 mm. c.r. stage, the linear pattern is lost completely.



Text-fig. 5. The numbers of villi on three series of cotyledonary plaques from FDE/15 (22 mm.), FDE/13 (25 mm.), and FDE/10 (18 mm.). The position of the cotyledons is indicated by those marked 1 being nearest the cervix and those marked 5 being nearest the tubo-uterine junction.

The primitive villi do not spring directly from the surface of the chorion, but arise from the summits of ridges whose long axes are approximately transverse to that of the chorion. These ridges, which taper away at the end of the row (Pl. 1, fig. 2), fit into depressions in the caruncle, into the bases of which open the crypts which will receive the villi (Pl. 1, fig. 3).

In every specimen, the primitive villi were expanded in their basal region parallel to the long axis of the row in which they were lying (Pl. 1, fig. 2). This condition persists for some time, but in later stages the base is relatively constricted compared with the more distal part of the villus (Pl. 1, fig. 6).

The earliest villi lie at an angle to the chorion with their long axes still in the same plane as the row from which they arise. This is probably due to their method of formation, which is not a simple outgrowth, but consists of an invagination of epithelium, at first downwards and then parallel to the surface, thus demarcating a process lying in the surface of the chorion (Pl. 1, figs. 4, 5). This process, by further growth, elongates and forms the primitive villus. The most primitive villi are short, curved, spike-like processes which soon become plump cylinders 1 mm. in length. None was observed to branch at its origin, but some (about 1 mm. long) can be seen to be bifid at their tips (Pl. 1, fig. 2).

It is probable that all the plaques on any one chorion are nearly the same age regardless of size. This implies that some plaques develop at a greater rate than others. This is probably especially true of those close to the foetus. The villi appear relatively widely spaced, along relatively widely separated rows. As the cotyledon gets older, the distance between the rows and the villi on them decreases. This may be due either to increase in the absolute size of the villi, or to more rows of villi, and more villi being added on each row to those already in existence. If the latter be the case, the newcomers rapidly reach the same stage of development as those already present. Increase in the age of the chorion leads for a time to increase in the number of villi present in any one cotyledon.

#### THE FORMATION AND GROWTH OF THE PLACENTOME

The placentome is formed as a result of the interlocking of the villi of the foetal (cotyledonary) component within the crypts which develop in the maternal (caruncular) component. The process commences with the appearance of the villi on the cotyledonary plaques; this occurs before, or at the same time as, the development of the crypts in the maternal caruncle (Table 2).

The caruncles in the non-pregnant uterus are elongated, oval, flattened masses with a smooth surface. Formation of crypts in the caruncle appears to commence in those caruncles nearest the foetus in the pregnant horn, and in those nearest the middle of the opposite horn. Crypts appear in those caruncles nearest the cervix before appearing in those nearest the tubal end of the horn.

The crypts first appear as small, localized ingrowths of maternal epithelium arranged in distinct rows related to the transverse linear depressions on the surface of the caruncle. Each ingrowth lies approximately opposite to a developing villus. The invaginated epithelium first forms a solid bud of cells with evidence of a more dense layer of connective tissue about it. There is also evidence of engorgement of the neighbouring capillaries and of invasion of this dense layer of stromal tissue by what are probably lymphocytes. Later the solid buds of epithelium break down in the centre, apparently due to necrosis (Hamilton & Harrison, unpublished), and the ingrowth of epithelial cells thus becomes a short narrow crypt. The crypt grows deeper due not only to a repetition at the depths of the crypt of the process that occurred earlier in the invasion of the villus, but also to growth in thickness of the caruncle. Use of the Schiff reagent, which, as Wimsatt (1951) found in the sheep allows the fate of the binucleated cells to be followed, shows that in *Dama* binucleated cells pass from the trophoblast to become intercalated in the crypt lining. Such migration occurs as soon as the villus has penetrated into the developing crypt.



Only a few cells migrate at first, and it seems that they die fairly soon after leaving the foetal epithelium as the majority of binucleated cells seen in the crypt wall show evidence of nuclear and cellular destruction.

When the embryo is 40 mm. in C.R. length (FD/37) the villi have penetrated all the flattened caruncles fairly deeply (Pl. 2, fig. 9). The pits for the villi are still arranged in distinct rows (Pl. 1, fig. 3); one row sometimes stretches right across the caruncle, passing transversely to the long axis of the caruncle (and usually that of the uterine horn). The surface of the caruncle is raised into numerous parallel ridges (about forty-five ridges across a caruncle 2 cm. in length (Pl. 1, fig. 3)). In the depressions between these ridges are found rows of crypts, within which lie the

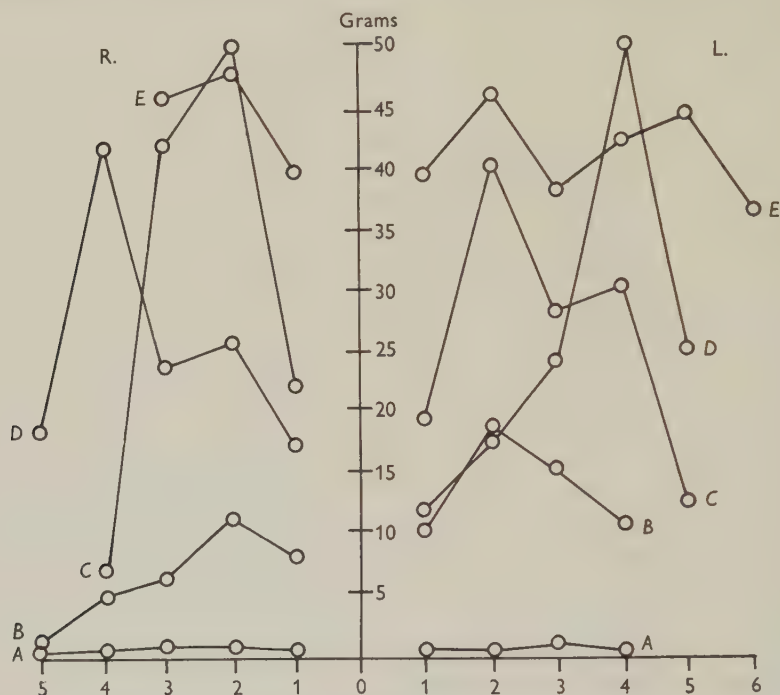
Table 2. *Details of animals recently pregnant*

Animal no.	Date killed	Embryo length	Remarks
FDE/11	2. xii. 52	Presomite embryo	No cotyledonary plaques, no caruncular crypts
FDE/17	2. xii. 52	1 mm. somite embryo	No plaques, no crypts
FDP/2	24. xi. 53	7 mm.	No plaques, no crypts
FDP/6	1. xii. 53		
FDE/4	25. xi. 52		
FDP/1	24. xi. 53	12 mm.	No plaques, no crypts
FDE/1	25. xi. 52	15 mm.	Primitive villi present on 2 or 3 plaques; no crypts
FDE/2			
FDE/3			
FDP/12	1. xii. 53	17 mm.	Villi, $\frac{1}{4}$ – $\frac{1}{2}$ mm. in length on four plaques near embryo. Transverse linear depressions on caruncles near embryo
FDE/5	25. xi. 52	18 mm.	{ Villi $\frac{1}{2}$ –1 mm. in length on all plaques, crypts $\frac{1}{2}$ –1 mm. in depth arranged linearly along the transverse depressions present on all caruncles
FDE/10	2. xii. 52		
FDP/9	1. xii. 53		
FDE/7	2. xii. 52	19 mm.	Villi present on all plaques and crypts found on all caruncles
FDE/6	2. xii. 52	20 mm.	
FDE/8	2. xii. 52		
FDP/8	2. xii. 53		
FDP/10	2. xii. 53		
FD/15	18. xii. 51	22 mm.	—
FDE/14	2. xii. 52	23 mm.	—
FDE/13	2. xii. 52	25 mm.	—
FD/6	15. xii. 51	27 mm.	—

villi. Estimations of the weight (Text-fig. 6) and size of the placentomes show that those placentomes nearest the centre of each uterine horn are significantly larger and heavier than the others.

The villi at this stage are between 6 and 8 mm. in length and are cylindrifform, tapering only slightly towards the tip. They average 1 mm. in diameter. Numbers of villi show evidence of branching; two to four branches of varying length may be seen arising from points close to the base of each villus. Occasionally branching occurs at the tip. The branches of one stem villus frequently occupy the same crypt at this early stage, but in many crypts secondary crypts are being formed at a slight angle to the main crypt. Not all the villi have become enclosed in crypts at these early stages; it is very common to find a fringe of villi floating freely in the space between the edge of the caruncle, the uterine mucosa and the reflected chorion. There frequently appear to be a greater number of villi on the cotyledon than there is room to receive in crypts in the caruncle.

At the time the foetus has reached the c.r. length of about 100 mm. the placentomes have become thicker, have risen away from the uterine wall to which they are attached by the narrowing base or pedicle. The increase in size appears to be due not so much to an increase in the quantity of maternal tissue, although this does increase *pari passu* with the growth of the placentome, but to an increase in the thickness of the villi (Pl. 2, fig. 10). These have all become much plumper,



Text-fig. 6. Diagram to indicate the weights of placentomes during pregnancy according to the position in the uterine horn. Those marked 1 are nearest the cervix, those marked 5 or 6 are nearest the tubo-uterine junction. *A* = FDE/46, non-pregnant; *B* = FDE/50, 108 mm. c.r.; *C* = FDE/72, 210 mm. c.r.; *D* = FDE/93, 240 mm. c.r.; *E* = FD/2, 380 mm. c.r.

although the covering epithelium retains its characteristic structure. Evidence of branching is now clearly apparent (Pl. 2, figs. 8, 10), and can be seen all along the villus, but most frequently in the basal third. The villi still retain their cylindrical form, but the tapering at the tip is more pronounced in the long villi. It will be seen from examination of Pl. 2, fig. 10, that the majority of the villi still enter the caruncle parallel to each other and in a vertical direction. In a number of caruncles of this and earlier stages of pregnancy a few small villi can be seen arising from the chorion at the edge of the caruncle. These villi are always less well developed than those in the centre of the placentome. It is possible that they either represent villi, formed at earlier stages, which have not been incorporated in the placentome, or that they represent new villi, which may later be incorporated in the placentome as the caruncle increases in size.

During the period covered by the increase of the length of the foetus from about 120 to 180 mm. there are further changes in the appearance of the placentome. Each placentome increases steadily in size, particularly in the half away from the uterine wall, but the placentome remains attached to the uterine wall by a pedicle which does not increase so greatly in size (Pl. 2, fig. 11). The result of this growth process is that the placentome eventually takes on an ellipsoidal form (Pl. 2, fig. 12). The ellipsoidal placentomes do not always remain with their long axes parallel to the long axis of the uterine horn, but tend to become arranged side by side with their long axes at right angles, or oblique to, the long axis of the uterine horn. Those placentomes which lie in the central part of each horn are still appreciably larger and heavier than those near the cervix or the tube (Text-fig. 6).

As a result of the growth changes the villi now enter the caruncle not only in a vertical direction from the foetal aspect, but also from the sides and from the under surfaces of the rolled-over edges of the caruncle (Pl. 2, fig. 12). The villi change in shape during this period of growth, and develop their final form. The branching of the previously plump, almost cylindrical or digitiform, villi results in the formation of a series of stem villi 5–6 mm. in length and 1–1.5 mm. in breadth, with from three to six long, narrow filiform secondary villi arising from them (Pl. 2, fig. 8). The secondary villi are 10–15 mm. in length, and measure 0.4 mm. in diameter at their origin. They taper steadily to end in a fine tip; each secondary villus may divide some 5 mm. from its tip to give rise to two or three short, fine, tertiary branches (Pl. 2, fig. 8). Each secondary branch is usually found within a crypt of its own, but the tertiary branches may remain in a single crypt.

Only a few specimens are available to cover the period when the foetus grows from the 300 mm. stage to term. The placentomes do not continue to increase in size as rapidly as they did in the earlier stages. There is, however, a tendency for the placentomes, except the accessory ones, all to become equally large (Text-fig. 6). Those placentomes near the cervix and the tube increase steadily in size and weight and approach, but never equal, the appearances of those in the central part of each horn.

#### THE INCREASE IN THE NUMBERS OF VILLI AND CRYPTS

In the early stages of development of the placentome it is relatively easy to withdraw the villi from the crypts. The chorion can then be stretched out on a piece of cork and the number of villi can be counted from photographs of each cotyledon. It is also possible to pluck the villi separately from the cotyledon under a dissecting microscope and count the total number obtained.

The crypts in the caruncles can then be counted by one of the methods described on p. 339. It must be emphasized that the following observations are concerned principally with the number of stem or *primary* villi and with the number of orifices of primary crypts seen on the *surface* of the caruncle. As soon as branching of villi occurs the appearances within the placentome become more complicated.

It has been shown that the total number of villi on the chorion is about 6000 to 8000 at the time when the crypts are starting to be formed in the caruncle (Table 3). In FD/37, at the 40 mm. C.R. stage (Pl. 2, fig. 13), the total number of villi on the chorion (8570) was greater than the total number of crypts (7190), and there



appeared to be a consistently higher number of villi than crypts in each placentome (Table 3). In FDE/50, at the 108 mm. c.R. stage, the chorion was unfortunately too macerated to allow counts of the villi. In another specimen at the 100 mm. c.R. stage only those caruncles in the left horn were examined, and the number of crypts in the four caruncles and one small accessory one was 3650 (Pl. 2, fig. 13).

Table 3. *The weights of the placentomes, the numbers of villi in the cotyledons and the numbers of crypts in the caruncles*

Placentome no. ...	Left					Cervix					Right				
	...	6	5	4	3	2	1	1	2	3	4	5	6	Tot	
FD/37 (40 mm.):															
Weight of caruncles (g.)	—	—		0.5	1.6	1.5	0.7	0.5	1.1	1.8	0.6	—	—	—	
No. of villi on cotyledons	—	—		790	1300	1150	990	950	1060	1350	980	—	—	8,5	
No. of crypts in caruncles	—	—		650	1080	1050	810	700	980	1210	710	—	—	7,1	
FDE/50 (108 mm.):															
Weight of placentomes (g.)	—		1.6	5.5	6.0	11.5	8	10	15	18.5	8	—	—	—	
No. of villi on cotyledons	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
No. of crypts in caruncles	—		140	850	980	1050	910	780	1130	1300	950	—	—	8,0	
FDE/51 (150 mm.):															
Weight of placentomes (g.)	—		8	35	22	14	9	18	19	28	17	11	6	—	
No. of villi on cotyledons	—		310	1660	560	530	380	540	430	710	490	430	131	6,1	
No. of crypts in caruncles	—		300	1200	730	590	210	600	560	680	580	550	200	6,2	
FDE/75 (202 mm.):															
Weight of placentomes (g.)	—	—		17	36	25	18	5	10	28	47	11	—	—	
No. of villi on cotyledons	—	—		570	980	870	770	290	330	630	800	290	—	5,5	
No. of crypts in caruncles	—	—		990	2200	2100	1100	450	720	1590	2300	400	—	11,8	
FDE/94 (240 mm.):															
Weight of placentomes (g.)		3	46	56	30	25	14	6	28	30	48	34	—	—	
No. of villi on cotyledons		250	3200	6120	2120	1700	1900	535	2250	1560	2450	1210	—	23,3	
No. of crypts in caruncles		240	3100	6090	1900	2070	2000	500	2300	2200	2860	1100	—	25,5	

In FD/37 the villi separated too easily from the crypts to allow accurate weighing of the placentomes.

In FDE/51, at the 150 mm. c.R. stage, the total number of villi (6171) almost equalled the 6200 crypts counted. In FDE/75, at the 202 mm. c.R. stage, the number of villi is somewhat low (5530), but the number of crypts has increased (11,850). At the 240 mm. c.R. stage there is a great increase both in the number of villi (23,275) and in the number of crypts (25,560) to figures three to four times those at the earlier stages. A number of individual placentomes from several other uteri pregnant with foetuses from 100 to 380 mm. c.R. length were also examined; the results are not included in Table 3 as only one or two placentomes from each uterus were examined, the remainder being cut up for histological examination. In general, the counts of the number of villi and crypts closely corresponded with those given in Table 3 for the respective foetal length. FD/2, pregnant with a 380 mm.

C.R. foetus, contained six placentomes in the right horn and three in the left. The nine placentomes were all of approximately similar size and weight. One of the largest placentomes contained 3900 primary villi and 3810 crypts.

#### DISCUSSION

The processes resulting in the formation of the placentome in *Dama dama* consist fundamentally of the development of villi on the cotyledonary plaques of the chorion, of the appearance of crypts in the maternal caruncle and of changes in form of the villi and caruncle after the villi have become enclosed in the crypts. The plaques appear on that aspect only of the chorionic sac lying against the caruncles and in a number equal to that of the caruncles. The caruncles are devoid of glands, and thus it could be argued that the cotyledons appear where the chorion is not separated from maternal tissue by products of glandular secretion. It could also be said, although there is yet no experimental evidence, that the presence of a given number of caruncles induces the appearance of an equal number of cotyledons.

Although the number of caruncles appears to be always equalled by the number of cotyledons at the time of the latter's first appearance, the size of the cotyledons and the number of villi each cotyledon possesses do not at first correlate. In the non-pregnant uterus those caruncles in the central part of each horn appear always to be larger than those near the tube or cervix, a relationship which also applies to the central placentomes of each horn during the first two-thirds of pregnancy. Yet on the chorion there is at first no such constant relationship between the size of each cotyledon, its position on the chorion, and the number of villi. It is as if the caruncles can induce the development of cotyledonary plaques on the chorion opposite to them but have no critical control over the size of the induced cotyledon.

It appears that the villi start to develop on the cotyledonary plaques in advance of crypt formation in the caruncles. Villi do not develop at random over the area of the cotyledonary plaque, but arranged in line, many lines parallel to one another and often at right angles to the long axis of the caruncle. The lines of villi are equidistant from one another, as are the villi along each line. This linear arrangement is not related to the vascular pattern of the chorion which has a reticular appearance all over the chorion. Turner (1878*a*), in fact, states that in *Rangifer tarandus*, the reticular appearance is due to raised ridges in the chorion with capillaries running in relation to these ridges, although he did not recognize the polygonal areas in *Cervus porcinus* (Turner, 1878*b*). Injection techniques have shown that the chorion is probably not responding to some vascular pattern on the caruncle since the surface vessels of the caruncle, at least, form an irregular network of subepithelial capillaries and lacunae.

It must therefore be assumed that the linear arrangement of the villi on the cotyledonary plaques is a species or generic characteristic of the Cervidae; it has been observed by us in young stages of *Elaphurus davidianus* and *Cervus elephas*, by Strahl (1906) in *C. elephas* and by Turner (1879) in *C. mexicanus*. The linear effect may be due to the action of the local induction mechanism causing villous formation or to some as yet unobserved structural characteristic of the chorion.

The number of villi in a cotyledon increases at first by new villi developing at both ends of each row of villi. New rows of villi, usually appearing on curved lines,

are apparently added to the tubal and cervical ends of the cotyledon. The number of villi in a cotyledon varies between 450 and 1700 at the time when all the cotyledons have appeared. The total number of villi on all the cotyledons is between 6000 and 8000 at this stage. A definite relationship between the size of the cotyledons, the number of villi on each cotyledon and the size of the caruncle obtains only when crypt formation is well advanced. There is evidence that occasionally there may be more villi than the caruncle can house in its crypts. These free villi have been observed forming a fringe about the developing placentome; it is possible that they become incorporated in the edges of the placentome later in development.

The villi develop as outgrowths from a distinct ridge; an ingrowth of chorionic epithelium occurs at one side of the future villus. The ingrowth tends to break down and the villus first appears at an angle to the chorion. It is probable that this sloping of the young villi is simply reflecting the manner in which the chorion is curved over the surface of the caruncle and that the villi tend at first to enter the crypts, not at right angles to the tangent of the surface of the caruncle, but parallel to one another and in a plane vertical to the long axis of the caruncle.

Early development of the crypts also occurs in rows, with the villi penetrating the crypts along the bottom of a series of parallel valleys on the surface of the caruncle. Thus it appears that the power of causing indentation of the caruncle is not limited to the villi alone, but is also possessed by the ridges from which the villi project. The linear arrangement of villi and crypts is lost between the 40 and 100 mm. c.r. stage. It disappears first in the larger placentomes and is replaced by an irregular or reticular pattern with the crypts becoming closely packed and each tending to have a polygonal outline.

The primitive villi are short, simple, straight and each is received in a crypt of its own. At an early stage bifurcation at the tips of the primitive villi can be observed. Later the villi become plumper and at about the 100 mm. c.r. stage reach their maximum diameter. Branching at the tips, and also lateral branching, is frequently observed at this stage. The total number of stem villi at this stage does not vary significantly above or below the total number found at earlier stages. From about the 190 mm. stage a distinct increase (to three to four times the original number) in the numbers of stem villi and primary crypts is observed. The villi are distinctly narrower at this stage and tend to reach their maximum length.

It appears, therefore, that the villi increase in number primarily by branching of villi already in existence. Additional crypts may be formed to incorporate within the growing caruncle those villi not originally included. Branching occurs at the tip or at the sides of a villus so that a stem villus may possess three to six growing secondary villi. Eventually the point of branching comes so close to the chorion that the stem villi are divided, giving rise to a discernible increase in the numbers of stem villi at about the 240 mm. c.r. stage. At this later stage short tertiary villi have been observed arising by bifurcation at the tips of the secondary villi.

Correlated with the branching of villi, growth of the caruncle occurs. At first those caruncles in the centre of each horn increase most rapidly in size; this is probably because this part of the uterine horn can dilate most easily, and injection techniques suggest that it may well have the greater maternal blood supply. Later



all the placentomes tend to become equal in size, presumably as more accommodation becomes available at the cervical and tubal extremities of the two horns. It has been noticed that the uterus in the Cervidae tends to increase in size during pregnancy by great extension of the anti-mesometrial wall of both uterine horns so that the uterus in the late stages of pregnancy becomes almost spherical (Harrison & Hamilton, 1952). This process would allow greater freedom for growth of placentomes at the tubal and cervical ends of the uterine horns.

The caruncles are able to accommodate increased numbers of villi not only by an increase in overall dimensions but also by a change in shape. At first the caruncles are sessile, elongated, flattened elevations and the crypts enter perpendicularly from the foetal aspect. The caruncles increase rapidly in depth and soon become raised on a thick pedicle like a 'loaf'; growth also occurs peripherally so that the crypts no longer enter the caruncle vertically, but at right angles to the tangent at the surface. Eventually the placentome takes on the form of an ellipsoid or an ovoid, raised on a narrow pedicle with the crypt-bearing area turned under all round its circumference. The outward growth of the caruncle results in a deepening of the crypts, and, since the villi are *pari passu*, decreasing in thickness, but increasing in number, there will be greater space available in the centre of the caruncle in which new crypts can be formed.

It will be understood that unrestricted outward growth of the caruncle would result in the formation of long, primary crypts with the secondary crypts opening from them, deep in the substance of the caruncle. In fact, observation of the surface of the caruncle at stages throughout pregnancy revealed the orifices of the secondary crypts coming nearer to the surface until they eventually reach it. At this stage the number of crypts counted at the surface of the caruncle increases significantly and the diameter of each crypt decreases. It is argued that this could only occur if the superficial caruncular tissue were steadily removed by attrition as the caruncle grew. Such a process is believed to occur and the resulting foetal-maternal relationship has been called diaphthoro-epithelio-chorial by Harrison & Hamilton (1952) in *Elaphurus davidianus*. The term has been criticized in that it does not describe a *functional* relationship in foetal-maternal exchanges, although it may reflect the attrition of the septal walls preventing compression of the base of the secondary villus by the growing caruncle. In *Dama*, at least, the argument developed above suggests that the attrition and probably partial destruction of the superficial septal tissue is essential during the important growth changes occurring during the development of the placentome. The process is closely associated with the prevention of the growth of a villus to immoderate and unnecessary length; it allows a considerable increase in the number of villi, and, incidentally, their total surface area, without greatly increasing the size of each placentome; and it ensures that the maximal part of each secondary villus is ensheathed in the physiologically active part of each crypt. Thus the process illustrates the adaptation of the 'oligo-cotyledonary' type of placenta for maximal physiological efficiency.

## SUMMARY

1. The appearance of the uteri of eleven non-pregnant *Dama dama* are described. *Dama* is monoestrous, and there is no evidence of delayed implantation from examination of 250 pregnant specimens collected at intervals over a period of three years.

2. In the non-pregnant uterus and for the first two-thirds of pregnancy the central caruncles, or placentomes, in each horn are the largest; the commonest number of caruncles or placentomes is 8 (range 6–12).

3. Villi develop in rows from the summits of ridges on cotyledonary 'plaques' which appear on the chorion opposite to the maternal caruncles from about the 10 mm. c.r. stage.

4. Crypts develop in the caruncle in rows corresponding to those of the villi, starting about the 18 mm. c.r. stage. The crypts appear in the floor of troughs between narrow ridges which usually lie at right angles to the long axis of the caruncle.

5. There is no distinct correlation at first between caruncular size, the number of crypts, size of the cotyledonary plaque and the number of villi on a plaque.

6. From about the 100 mm. c.r. stage the linear arrangement of the villi and crypts disappears. A close correlation eventually develops between the size of the placentomes, the number of crypts and the number of villi.

7. The total number of primary villi is at first between 6000 and 8000; there is an increase to about four times this number by the 250 mm. c.r. stage, mainly due to division of villi already in existence.

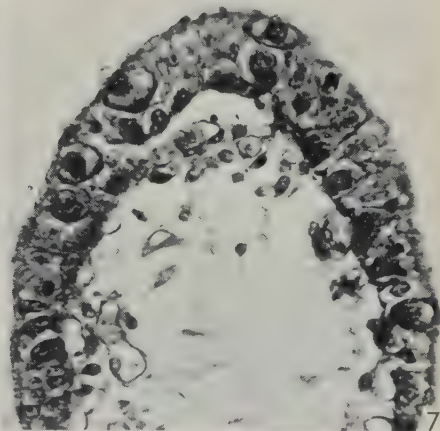
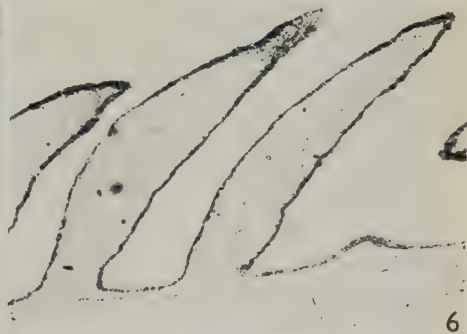
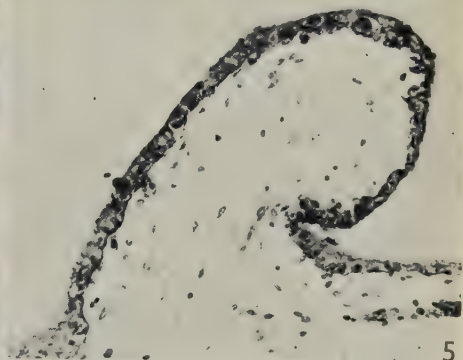
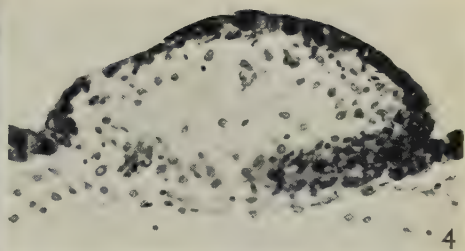
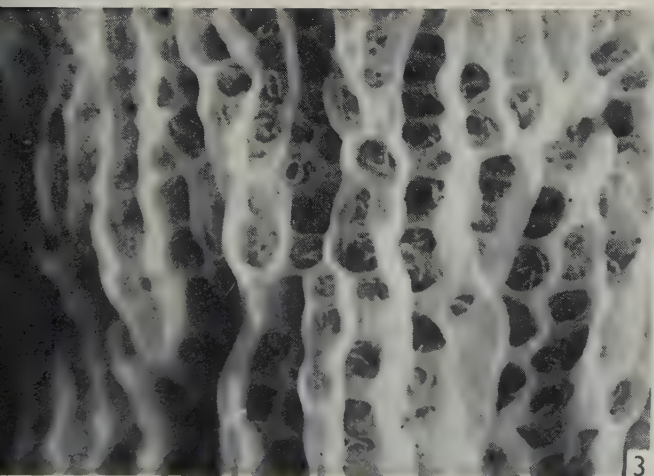
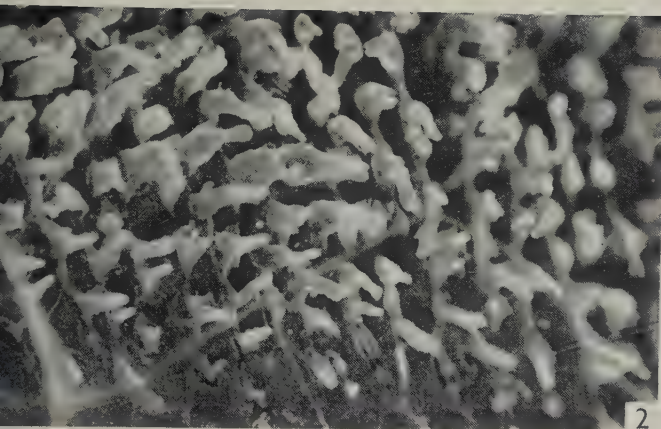
8. There is a progressive change in form and size of the villi, in the size of the crypts, and in the shape of the placentome throughout pregnancy.

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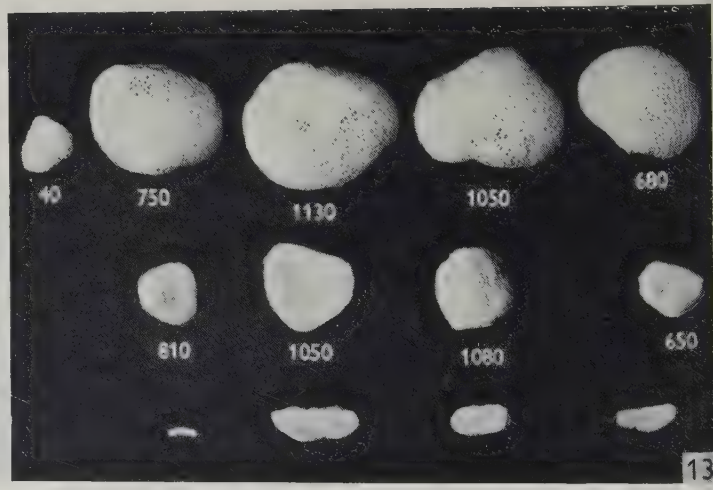
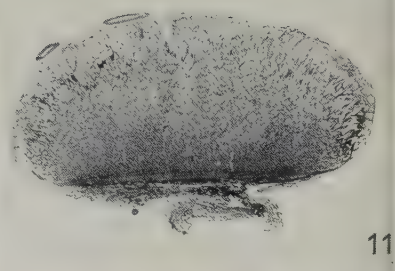
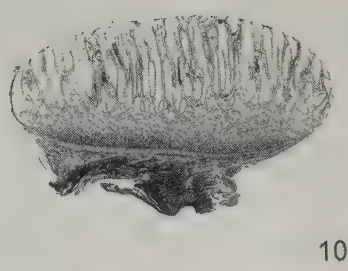
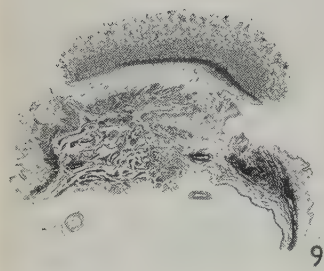
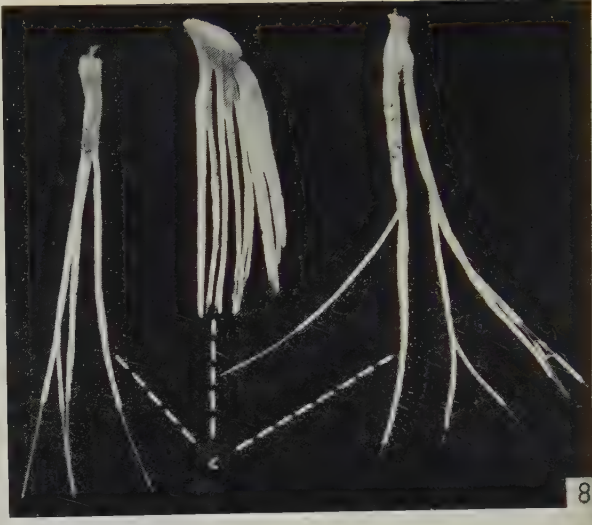
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## EXPLANATION OF PLATES

### PLATE 1

- Fig. 1. A low-power photograph of a cotyledonary plaque (FD/15), showing the developing villi. The rows of villi can be seen, and it is clear that the linear arrangement does not correspond with the radiating arrangement of the chorionic vessels.  $\times 3.3$ .
- Fig. 2. Photograph under higher power of the lower edge of the cotyledonary plaque in Fig. 1. It shows the young villi developing at the periphery of each row, and also occasional branching of villi.  $\times c. 23$ .
- Fig. 3. Photograph of the surface of a maternal caruncle from FD/37, showing the linear arrangement of crypts.  $\times c. 23$ .
- Fig. 4. Photograph of a section through a developing villus from FD/7.  $\times 170$ .
- Fig. 5. Photograph of a section through a developing villus of FD/18.  $\times 150$ .
- Fig. 6. Photograph of a section through a row of developing villi from FD/18.  $\times 32$ .
- Fig. 7. Photograph of a section through the tip of a villus from FD/18, showing binucleated cells.  $\times 360$ .

### PLATE 2

- Fig. 8. Photographs of villi withdrawn from their crypts, from several pregnancies at different stages: (a) from FDE/50 (108 mm.); (b) from FDE/51 (150 mm.); (c) from FDE/94 (240 mm.).  $\times c. 2.5$ .
- Fig. 9. Photograph of a longitudinal section through a placentome from FD/18 (30 mm.).  $\times 1.2$ .
- Fig. 10. Photograph of a longitudinal section through a placentome from FD/28 (100 mm.).  $\times 1.3$ .
- Fig. 11. Photograph of a longitudinal section through a placentome from FD/3 (270 mm.).  $\times 1.1$ .
- Fig. 12. Photograph of a longitudinal section through a placentome from FD/44 (380 mm.).  $\times 1.3$ .
- Fig. 13. Photographs of a series of caruncles from: lower row, four caruncles from the left horn of FDE/12 (non-pregnant); middle row, four caruncles from the left horn of FD/37 (40 mm.); upper row, five caruncles from the left horn of FDE/49 (100 mm.).  $\times 0.5$ .

## OBSERVATIONS ON HUMAN CHORIONIC VILLI USING THE ELECTRON MICROSCOPE

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The acquisition recently of a very fresh human chorionic sac containing a 6 mm. c.r. length embryo (H. 543), through the good services of Mr O. Lloyd, F.R.C.S., of Addenbrooke's Hospital, Cambridge, has enabled us to make some observations on the structure of the chorionic villi using the electron microscope.

### METHODS

For electron microscopy chorionic villi were fixed in 2% osmic acid for 24 hr., washed in several changes of distilled water, and dehydrated. In previous observations on the electron microscopy of embryonic material (Hughes, unpublished) no difference in the quality of fixation has been observed between tissue fixed in buffered osmic acid (Palade, 1952) and in the unbuffered solution. From absolute alcohol the villi were transferred to a mixture of half absolute alcohol and half butyl methacrylate monomer, and after 24 hr. to the pure monomer. A day later portions of the material were placed in gelatin capsules, together with the monomer to which was added a small quantity of benzoyl peroxide, as catalyst for the polymerization of the methacrylate. The capsules were then placed in an incubator at approximately 45° C. for several days, after which the gelatin envelope was dissolved in hot water and the polymerized block mounted on a Cambridge Rocker microtome adapted for fine section cutting. Sections were cut as thin as possible with glass knives and were mounted on copper grids coated with nitrocellulose. They were examined under the Siemens electron microscope in the Cavendish Laboratory, thanks to the courtesy of Dr V. E. Cosslett and the staff of the Electron Microscope Group. The thickness of the best sections was judged to be of the order of  $0.03\mu$ . The initial magnifications of the negatives were either  $\times 4500$  or  $\times 8000$ . Subsequent enlargements were made at a final magnification of 10,000–26,000 diameters.

In addition to the sections for electron microscopy, series of sections of 4, 6 and  $10\mu$  in thickness, and stained with haematoxylin and eosin, Heidenhain's iron haematoxylin, Mallory, and Azan were available for observation under light microscopy.

### OBSERVATIONS

The syncytiotrophoblast, the cytotrophoblast, the mesodermal core and the capillaries of the villi have been studied both by light and electron microscopy.

*Syncytiotrophoblast.* As is well known the syncytium covering the chorionic villi in the central mass of the developing placenta shows certain differences from that which covers the trophoblastic cell columns and lines the trophoblastic shell. Our material prepared for electron microscopy was taken from villi in a region about midway between the differentiating basal and chorionic plates. It is, therefore, villous (or 'resorptive', Grosser, 1927) syncytium with which our observations are principally



concerned. We are not able to comment effectively on the differences alleged by such investigators as Grosser (1927) and Florian (1928) to exist between such syncytium and that related to the cytotrophoblastic shell and which has been called 'implantation' or 'proliferative' syncytium, or plasmodium. From a study of the sections prepared for light microscopy, however, there do not seem, in fact, to be sufficient grounds for the division of the syncytium into these two types, a conclusion similar to that which was reached by Wislocki & Bennett (1943).

In the stained sections the syncytium can be seen to possess a cytoplasm darker than that of the cytotrophoblastic cells; its nuclei are smaller, more irregular and usually have a greater affinity for haematoxylin. In our sections we have not noticed any sign of the shadows of former cell boundaries such as have been reported by Hamilton & Gladstone (1942) in the syncytium of the chorionic villi in a much younger embryo. In the syncytial cytoplasm the 'foamy' appearance described by many workers and stressed by Wislocki & Bennett (1943) can frequently be seen. In certain regions, mainly on the smaller villi, less frequently in the covering of the larger villous stems and rarely on the chorionic plate more or less marked cytoplasmic vacuolization can be seen in the syncytium. In many regions, too, a 'brush border' (*Bürstenbesatz*) can be identified. The brush border is very variable. So far as observation with ordinary microscopy can take one (for the dimensions go beyond the limits of resolution) there appear to be areas of the syncytial surface with no sign of a brush border. Adjacent areas, however, may possess either a well-defined one or a complicated and variable series of protoplasmic processes most readily studied in thin ( $3-5\mu$ ) sections, which are not well enough ordered, or regular enough, to justify the use of the term brush border. These last regions undoubtedly correspond to the areas 'which exhibit pale, ill-defined streamers or delicate fronds of stippled cytoplasm' in the syncytial surface of the placental villi in the rhesus monkey and in the human which have been described and discussed by Wislocki & Bennett (1943). Indeed, careful examination of the syncytial surface bordering the inter-villous space leads to the conclusion that the surface is never completely smooth if fixation has been early and adequate, and that a whole spectrum of appearances can be presented from a mere shagginess to a tall brush border of up to  $2\mu$  in height. To what extent the differences fluctuate in life is a problem that cannot be decided from a study of fixed material, but there is no appearance of a fixed structure in the syncytial surface that could prevent any part of it from producing processes or a brush border. The brush border and the irregular processes seem to stain, in haematoxylin and eosin sections, less deeply than the general cytoplasm of the syncytium, but thin paraffin sections suggest that this difference is not due to any histochemical distinction between the two.

Under the electron microscope also the appearance of the syncytiotrophoblast varies in different regions, both at the surface and within. Where the brush border is not present (Pl. 2, fig. 2) the cytoplasm consists mainly of densely packed vesicles about  $0.3\mu$  in diameter. This vesicular texture extends to the surface of the syncytiotrophoblast, in a marginal zone beyond a surface membrane. This is the zone which in other regions may be occupied by the brush border. The latter, where present, is made up of irregular filaments  $40-100\text{ m}\mu$  in diameter and extending in places up to  $3\mu$  beyond the surface membrane. Some of these filaments terminate in globular

expansions (Pl. 2, figs. 4, 5) which may be as much as  $0.3\mu$  across. Scattered along the course of the filaments are tiny granules near the limits of resolution of the microscope. Some of these seem to project beyond the general surface of the filaments. There are no signs of basal granules in the region of attachment to the surface membrane.

When the surface of the syncytiotrophoblast has a brush border, the cytoplasm just beneath is much vacuolated (Pl. 1, fig. 1). These vacuoles vary much in size; the largest are about  $3-4\mu$  across. Small vacuoles in this position are still seen underneath areas where the border has an intermediate type of structure (Pl. 2, fig. 3). Appearances suggest that vacuoles may be formed by the dilation of the protoplasmic vesicles.

Two other types of inclusion are also seen within the cytoplasm. Of these, one consists of abundant ovoidal bodies  $0.1-0.4\mu$  across. These clearly correspond to mitochondria. The other type is made up of fewer and larger granules which are densely impregnated with osmium and are  $1\mu$  or more in diameter. These larger osmiophil granules correspond to the lipid droplets which Wislocki & Bennett (1943) regard as consisting of placental steroid hormones.

The nuclei of the syncytiotrophoblast are vesicular in form, with most of their contents aggregated either beneath the nuclear membrane or in central clumps. They are clearly distinguishable in texture from those of the cytotrophoblast.

*Cytotrophoblast.* Our electron microscopic observations on the cytotrophoblast were made almost exclusively on the Langan's cells of the villi. Consequently, no descriptions will be given of the appearance in the material prepared for light microscopy of the trophoblastic shell or the cytotrophoblastic cell columns. It should perhaps be mentioned, however, that, in fortunate sections, the Langan's cells of the established villi can be traced towards the cytotrophoblastic cell columns and can be seen gradually to take on the characteristics of the latter. As the chorionic sac had been separated from the decidua we can make little effective comment on the cells of the trophoblastic shell. In Langan's layer only rare mitoses can be seen. Such evidence of cell division is more frequent in the cytotrophoblastic cell columns. In our specimen the cells of Langan's layer of the cytotrophoblast are usually so arranged that they form a continuous lining to the overlying syncytium. There are, however, regions in which the Langan's cells are discontinuous and the underlying mesodermal core of the villus is separated from the inter-villous space only by syncytium. This process of gradual disappearance or, at least, marked diminution in the number of the cytotrophoblastic cells, is one that progresses rapidly in stages older than that with which we are concerned. Other material available to us (Hamilton & Boyd, unpublished) suggests that there is little or no degeneration of the cells of Langan's layer; most of the cells seem eventually to become transformed into syncytium and possibly into cells of the mesodermal core. A few, however, persist in isolation and can still be identified in full-term placentae.

The cytoplasm of the Langan's cells is distinctly less basophilic than that of the syncytium, and consequently the cells are readily distinguishable in sections by their pale-staining. In general, too, the cytoplasm of the cytotrophoblast is less vacuolated than the syncytium, though the vacuolation is most variable. The nuclei of Langan's cells are distinctly larger than those of the syncytium, and are also more

palely staining with haematoxylin, though, as with the vacuolation in the cytoplasm, there is some variability.

Under the electron microscope the cytotrophoblast is clearly distinguishable from the syncytiotrophoblast by certain features in addition to the presence of sharply demarcated cell boundaries. Thus the cytoplasm is of a more even texture, the mitochondria have a more definite boundary layer (Pl. 3, fig. 6), and the nucleoplasm is much more uniform (Pl. 3, fig. 7). The nuclei of the cytotrophoblast correspond closely in appearance with other embryonic nuclei which we have observed in electron micrographs.

*Mesodermal core of villi and capillaries.* The stroma of the villi contains a loose mesenchymatous connective tissue embedded in which are thin-walled capillaries, possessing diameters of  $5\text{--}25\mu$ . At rare intervals, larger blood vessels can be found. With suitable staining the collagenous fibres of the connective tissue stroma apparently form a very loosely woven network. At the boundary between the stroma and Langhan's layer collagen fibres can be found to terminate in intimate relations with the cytotrophoblastic cells and occasionally it appears as if the collagen surrounds those cells.

Our studies on the mesodermal core in electron micrographs are at present limited to observations on the collagen fibres within the tenuous mesenchyme.

The collagen at this stage is mainly in the form of single macromolecular fibrils, in each of which the repeat pattern is seen as a row of dots (Pl. 3, fig. 9). The dots are separated by distances of the order of  $600\text{ \AA}$ , which correspond to the spacing within adult rather than embryonic collagen fibres (Randall, Frazer, Jackson, Martin & Worth, 1952). The fibrils are often arranged in parallel bundles, in which the single filaments are distinct. At the edge of the mesodermal core, these fibrils are seen to enter the substance of the cell walls of the cytotrophoblastic cells, and form a relatively dense network within the cell margin. This same arrangement is seen in the endothelial cells of a capillary within the mesodermal core (Pl. 3, fig. 8).

#### DISCUSSION

The most striking feature of the electron micrographs of the placental villi is, perhaps, the brush border of the syncytium. The possession by the syncytium of this brush border, or *Bürstenbesatz*, has frequently been recorded. Kastschenko (1885), in his description of the syncytium, writes that 'der freie Rand ist gewöhnlich von Wimpern besetzt'. As Grosser (1927) states, this appears to be the first description of the brush border, and Amoroso (1953) is mistaken in attributing the first observations to Minot. There are many later references to the brush border (Bonnet, 1903; v. Lenhossek, 1902; Marchand, 1903; Hofbauer, 1905; Jung, 1908; Herzog, 1909; Johnstone, 1914; von Möllendorff, 1921; Stieve, 1926; Greenhill, 1927). The observations up to this last date were summarized by Grosser who wrote 'An der äusseren Oberfläche trägt das Syncytium einen Besatz aus starren Stäbchen oder Härchen (Stereocilien) auch als Bürstenbesatz bezeichnet...der Besatz is nicht immer nachweisbar, und seine Erhaltung ist nicht an besonders gute Fixierung gebunden'. More recently, Wislocki & Bennett (1943) have given a much fuller account of the surface of the syncytium than was previously available. From their own studies, and from a survey of the literature, these investigators consider that the so-called brush



border is very variable in structure and that it is inconstant in its occurrence and distribution within any given placenta. They consider that there is some justification to assume that the syncytium is unstable and that its contours must be subject to fairly constant modification, and they suggest that the syncytium, temporarily at least, may be less mobile where the brush border is most apparent and complete. On the other hand, in those regions where fronds and streamers of the syncytial surface occur the cytoplasm may be in a greater state of flux. From considerations such as this Wislocki & Bennett suggest that the various surface irregularities of the syncytium are implicated in the taking up of fluid and nutriment from the blood in the intervillous space.

Recent studies by means of the electron microscope have shown that more than one type of structure in various tissues has been included under the heading of a brush border. In the cells of the intestinal epithelium, as shown by Bretschneider's study (1949) of *Ascaris*, the brush border consists of a dense pile of parallel filaments; in the kidney tubules (Sjöstrand & Rhodin, 1953) the brush border consists of densely arranged cylindrical 'ducts' which are closed towards the tubular lumen. In human chorionic villi, the present work demonstrates that the brush border is made up of individual filaments, but in an irregular arrangement with little or no tendency towards alignment in parallel tufts.

In the chick chorio-allantoic membrane, Murphy & Bang (1952) have shown that from the surface fine filaments ('microvilli') project which hypertrophy in material infected with Newcastle virus (Bang, 1952), when they often develop 'balloons' at the tip. These microvilli are reminiscent of the corresponding structures in human chorionic villi, though in the chick membrane they are more sparsely distributed. Again, somewhat similar projections are seen in the yolk-sac of the guinea-pig (Dempsey, 1953). These are spherical in early stages, but later become cylindrical. This author considers these structures to be absorptive in function. The correlation between the distribution of superficial vacuoles and the presence of these fully developed microvilli in the human syncytiotrophoblast strongly suggests the same conclusion.

The evidence that the surface of the syncytioblast is pinocytic (Lewis, 1931) has been already discussed by Wislocki & Bennett (1943). To this may now be added our observations that the presence of relatively small vacuoles close beneath the surface of the syncytiotrophoblast is always correlated with a fully developed brush border.

Presumably the distribution of microvilli in limited areas is due to their cyclic formation and disappearance; this interpretation is supported by the appearance of areas of an intermediate type such as is shown in Pl. 2, fig. 3, though whether this represents a stage in the development or the degeneration of microvilli cannot yet be decided.

It is clear, however, that the brush border of the chorionic villi is a less elaborate and less permanent structure than that of intestinal and renal epithelia, and we suggest that the term be abandoned for the syncytiotrophoblastic surface fringe. The individual elements of this, in those regions where order and structure are apparent, are adequately described as microvilli, the term introduced for comparable structures in other embryonic membranes by Murphy & Bang (1952).

# SUMMARY

1. Observations on the structure of human chorionic villi, using electron microscopy, are reported.

2. In areas possessing the so-called 'brush border' the surface of the syncytium of the villi shows filaments (microvilli) of up to  $3\mu$  in height and with diameters of  $40\text{--}100\text{ m}\mu$ . The microvilli may terminate in globular expansions which may be as much as  $0.3\mu$  across.

3. The cytoplasm of the syncytium has a vesicular structure in which vacuoles, mitochondria and large osmiophil granules can be identified. The presence of many vacuoles beneath the syncytial surface can be correlated with the presence of microvilli, and it is suggested that these areas are regions of absorption, possibly of a pinocytic nature. The osmiophil granules correspond to the lipoid droplets of the syncytium.

4. Differences between the nuclei of the syncytium and of the cytotrophoblast are noted.

5. The early collagen fibrils of the mesodermal core of the villi are described.

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### EXPLANATION OF PLATES

Figs. 1-9 are electron micrographs of areas in sections of villi from a human chorionic sac which contained an embryo of 6 mm. c.r. length. The initial magnifications of the prints were from 11,000-26,000 diameters. The distance corresponding to a micron in the section is given for each figure.

#### PLATE 1

Fig. 1. Tip of a villus, showing well-developed microvilli at the surface, with numerous vacuoles within the syncytium. One syncytial nucleus is shown near the lower margin of the figure.

#### PLATE 2

Fig. 2. Edge of a villus with amorphous cytoplasm at the margin. Mitochondria and lipid granules are seen within the syncytium. No vacuoles are present.

Fig. 3. Edge of a villus where the syncytial margin is intermediate in form. Some small vacuoles are present beneath the border.

Figs. 4, 5. Microvilli at higher magnification. They are irregular in arrangement and some terminate in globular expansions.

#### PLATE 3

Fig. 6. Section through a villus extending from the syncytium (top left) through a cytotrophoblastic cell into the mesodermal core (bottom right). Notice fine collagen fibres inserted within the marginal zone of the cytotrophoblastic cell and the mitochondria within this cell.

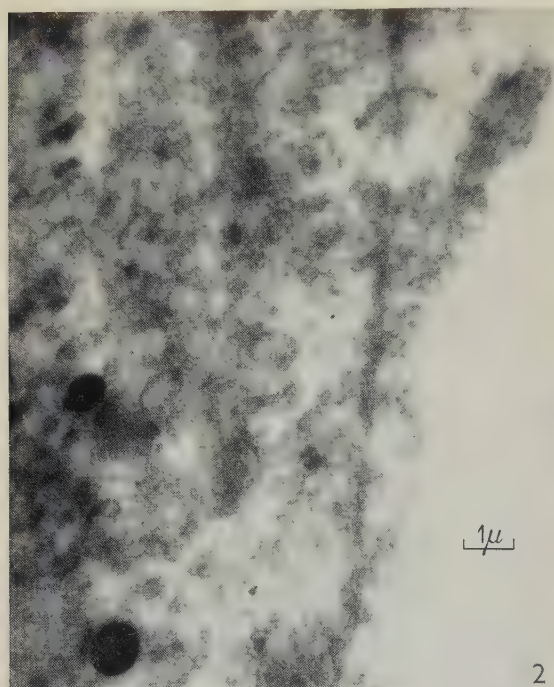
Fig. 7. Section through a cytotrophoblastic cell showing the nucleus and nucleolus. Notice the uniform texture of the nucleoplasm in contrast to that of the syncytial nuclei at this stage (Figs. 1 and 3).

Fig. 8. Section through a blood vessel within the mesodermal core of a villus. On the outside the collagen fibrils of the core are inserted within the marginal zone of the endothelial cells.

Fig. 9. Collagen fibrils within the mesodermal core. The dots within each fibril are separated by about 600Å. In the original negatives these dots are very clear indeed.



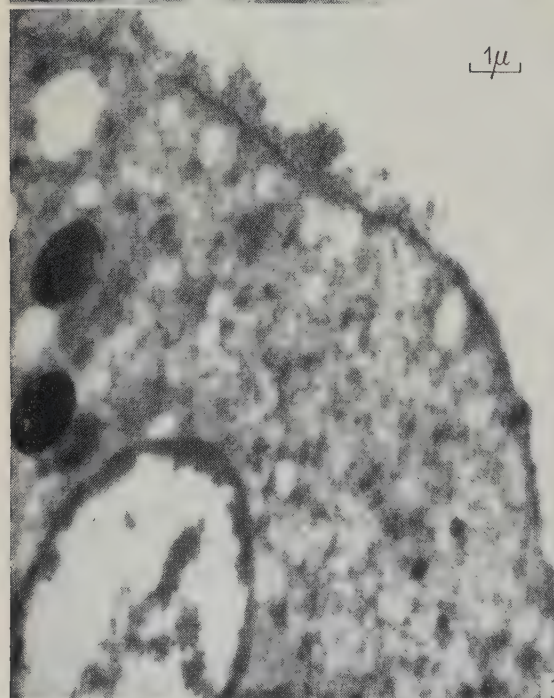




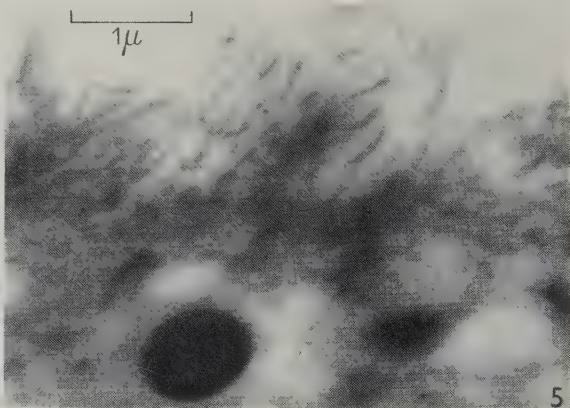
2



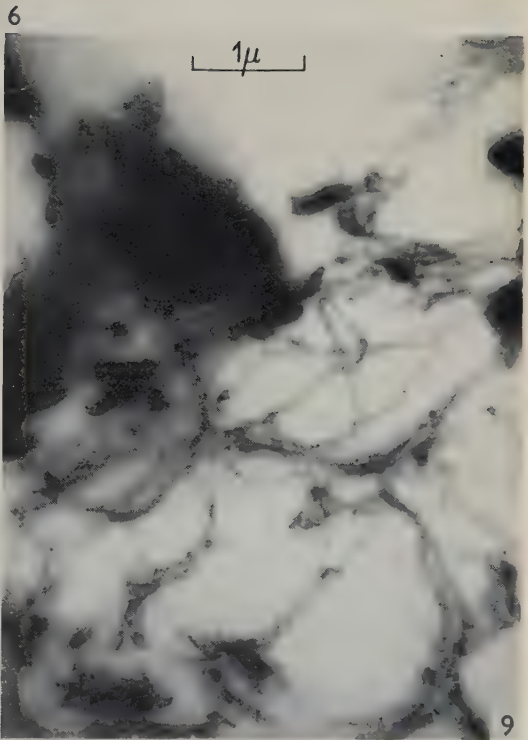
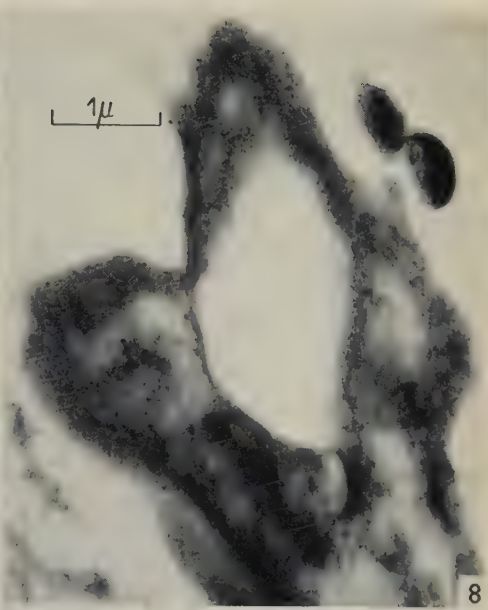
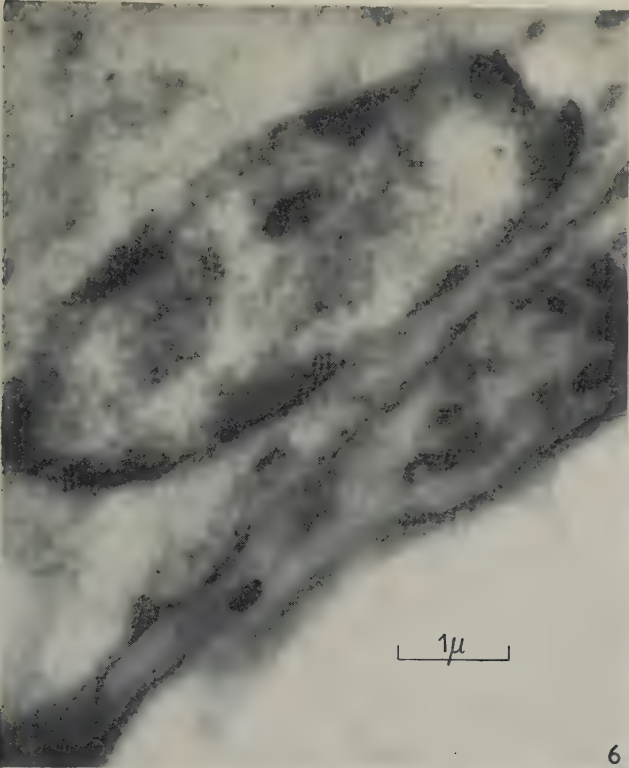
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3



5







# THE STRUCTURE AND FUNCTIONS OF FIBROCARILAGES WITHIN VERTEBRATE JOINTS

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MacConaill (1932) has summarized the various theories that have been advanced in the past to account for the presence of fibrocartilages within certain vertebrate joints, and has himself suggested a new explanation—that they act as ‘thrust-pads’, ensuring adequate lubrication of the bearing surfaces. While these structures doubtless assist lubrication in a joint that possesses them, it does not necessarily follow that this is their only function.

In the present investigation, particular attention has been paid to the type of movement occurring at joints containing fibrocartilages, rather than to the form of the articular surfaces. It is concluded that fibrocartilages are present in those articulations where some of the movements characteristic of ball-and-socket and condyloid joints (that is, flexion and extension, abduction and adduction, or axial rotation) are combined with the movements characteristic of plane joints (that is, to-and-fro gliding of one bone without any angular displacement). This theory is in many respects a return to that of Parsons (1900).

## MATERIAL

Sixty vertebrate joints containing fibrocartilages have been dissected. Of these the following have been selected for special comment:

The occipito-atlantal joints of a skate (*Raia brachiura*).

The cervical intervertebral joints of the pigeon (*Columba palumbus*).

The knee of the tortoise (*Testudo graeca*).

The knee of the rat (*Rattus norvegicus*).

The knee of the fruit-bat (*Pteropus aegypticus*).

The superior tibio-fibular joint of the Tasmanian devil (*Sarcophilus harrisii*).

The ankle joint of the Australian opossum (*Trichosurus vulpecula*).

The so-called ankle (mid-tarsal) joint of the fowl (*Gallus domesticus*).

Passing reference will be made to certain other vertebrate joints that exemplify particular features.

## OBSERVATIONS

Only the observed movements at each joint are considered here; no attempt is made to describe the exact form of the articular surfaces.

### *The occipito-atlantal joints of the skate*

In this bilateral joint there is a thin, biconcave, fibrous plate, perforated in the centre and containing a small amount of fibrocartilage at its periphery, situated between each occipital condyle and the corresponding ovoid facet on the atlas (Davies, 1948). Here two movements can occur: flexion and extension of the head,

during which the menisci remain fixed upon the atlas, and rotation of the head, during which the condyles together with the menisci slide dorso-ventrally upon the atlantal surfaces.

#### *The cervical intervertebral joints of the pigeon*

Between the rounded articular surfaces on the anterior and posterior zygapophyses of the cervical vertebrae of the pigeon, thin, fibrous menisci containing scattered cartilage cells are present. In these joints there is gliding of one bony surface upon the other when the bird laterally flexes its neck. During dorso-ventral flexion of the neck the anterior zygapophyses rotate upon the posterior. Very little axial rotation can occur between the heterocoelous vertebral bodies.

#### *The knee of the tortoise*

In this unusual joint the lateral meniscus is well developed but there is no medial meniscus. The medial femoral condyle is rounded, fitting into a concavity on the tibia. The movements are similar to those described by Haines (1942) in *Emys blandingii*. Flexion and extension occur but are of limited range; in axial rotation the femur spins about a vertical axis passing through the centre of the medial femoral condyle.

#### *The knee of the rat*

The menisci in rodents have been described in detail by Pederson (1949). In adult rats there are ossific centres in the anterior parts of both menisci and sometimes a third in the posterior part of the lateral meniscus. These centres, the lunulae of Pearson & Davin (1921), have been recorded in a number of other species, and their presence has been confirmed by the author in the squirrel, mouse, slow loris, iguana and Varanus lizard. Details of their incidence are given in Table 1, together with measurements of the angle between the upper and lower surfaces of the menisci in the region of the lunulae. The movements possible at the rat's knee resemble those in man, but flexion is so free that in fresh material the tibia and femur actually cross.

#### *The knee of the fruit-bat*

The statement by Parson's (1900) that the knee of the fruit-bat is exceptional among the mammals in lacking semilunar menisci is confirmed. As he pointed out, there is no axial rotation at the knee in this species, movement being limited to flexion and extension.

#### *The superior tibio-fibular joint of the Tasmanian devil*

A number of marsupial species show a semilunar meniscus between the upper ends of the tibia and fibula (Barnett & Napier, 1953). *Sarcophilus* is a good example. This joint allows rotation of the fibula about its long axis and also forward or backward sliding of the fibula upon the tibia (Haines, 1942). Since the plane of this joint is approximately in the long axis of the limb the pressure across the articular surfaces must be very small.



*The ankle of the Australian opossum*

Most marsupials possess a semilunar cartilage between the fibular malleolus and the talus (Parsons, 1900); in the Australian opossum it is unusually well developed. In this and related species the fibula rotates freely about its long axis, and the lateral surface of the meniscus provides a concavity in which the rounded malleolus can turn. During flexion and extension, a gliding movement takes place between the lateral surface of the talus and the medial surface of the meniscus.

*The 'ankle' of the fowl*

As previously recorded (Barnett, 1954), there is a striking resemblance between the ankle of most birds and the knee of mammals, both joints containing paired fibrocartilaginous menisci. Since these two articulations are in no way homologous, the resemblance must be entirely due to similarity of function. In the ankle of the fowl the most obvious movement is that of flexion and extension, but in addition axial rotation can occur by a forward sliding of the medial tibial condyle upon the corresponding tarsal facet together with a backward sliding of the lateral condyle.

Table 1. *The angle between the upper and lower surfaces of certain knee menisci, and the incidence of lunulae*

Species	Lateral meniscus		Medial meniscus	
	Anterior horn (degrees)	Posterior horn (degrees)	Anterior horn (degrees)	Posterior horn (degrees)
Mouse	45*	30	45*	25
Rat	45*	40†	45*	25
Squirrel	48*	30	30	25
Nycticebus	48*	25	45†	25
Varanus	45*	25	25	25
Iguana	50*	45*	35	30

\* Lunula constant.

† Lunula occasionally present.

## DISCUSSION

*Form and microscopic structure*

Typically, intra-articular fibrocartilages are deeply concave on one side and almost plane on the other, but sometimes (for example, in the ankle of the fowl) they are biconcave. In a weight-bearing joint the disc is commonly perforated (the knees of some lizards and birds being exceptional in this respect), giving rise to a meniscus with paired horns that are usually attached to one or other of the articulating bones.

The general term 'fibrocartilage' has been used in referring to the structure of intra-articular menisci and discs, though it is somewhat misleading since many are composed almost entirely of fibrous tissue with only a very slight admixture of cartilaginous matrix. In others, bony lunulae are constantly present. These were stated by Harris (1934) to represent a degenerative change, the meniscus having outgrown its source of nutrition. Evidence against this view is the fact that much larger cartilages in other species remain entirely unossified. It would appear from the present study that lunulae occur in those fibrocartilages where the upper and lower surfaces are steeply inclined to one another. In Table 1, several menisci that contain

lunulae are listed, together with an estimate of the angle between these surfaces. The critical angle is about 45 degrees; wherever the steepness of a fibrocartilage exceeded this figure a centre of ossification was present. Probably the bone serves to prevent undue compression of a wide-angle wedge, thus maintaining a synovial film of optimal thickness between the moving surfaces.

### *Functions*

Almost all the joints in this series allow sliding of one bone upon another. The only two exceptions are the knee of the fruit-bat and the medial half of the knee of the tortoise, both of which lack fibrocartilages. One may conclude that it was the necessity for a sliding movement, to co-exist with flexion and extension, that resulted in the evolution of fibrocartilages. In bicondylar joints this sliding movement occurs during axial rotation.

Wood Jones (1944) has stated: 'It is difficult to believe that Nature could not fashion the articulating bones to make an accurately fitting joint, and so adopted the plan of inserting washers to complete the adjustment.' The explanation here put forward depends upon the fundamental difference between an 'ovoid' articulation (such as a ball-and-socket or condyloid joint) and a plane articulation. In the former, one convex surface (the entering surface) fits into a corresponding concavity (the receiving surface). Stability is maintained by the tonic contraction of surrounding muscles, the tension in the joint ligaments and the congruity between the articular surfaces. The more rounded the entering surface and the deeper the receiving surface the more stable does the joint become (Sullivan, 1922).

An ovoid joint could conceivably have a well-rounded entering surface and a receiving surface that is almost flat; the customary movements would take place, but in addition a to-and-fro gliding of the upper bone upon the lower could occur, as in a plane joint. This additional movement would be obtained at the expense of stability. Although no joints exist in nature that exhibit this form of instability, Adams (1953) has reported that it may occur in artificial joints fashioned by the surgeon. In the conventional form of cup arthroplasty of the hip, the new acetabulum is much flatter than the femoral head. Adams states that, as a result, '... at a certain point in the movement the femoral head will suddenly slip into another part of the cup'.

The interposition of a movable disc in the interval between a convex entering surface and a flattened receiving surface allows a to-and-fro gliding to take place without making the whole articulation unstable, but its effectiveness will depend upon the ligaments or muscles attached to the disc. These must steady it on one bone during angular displacement but permit free movement during gliding of one bony surface upon another. Fibrocartilages are never loose within the joint but always have tendinous or ligamentous fibres inserted into their periphery.

Thus a combination of movements as discussed above, together with the necessity for a stable articulation, suffices to account for the evolution of intra-articular fibrocartilages. Three additional functions have been noted in the present series, however:

In joints where the lower bony surface is convex instead of plane, the disc must necessarily be biconcave, as in the knee of the rat. This form of fibrocartilage allows

a greater range of joint movement, for not only can each femoral condyle flex and extend within the concavity presented by the upper surface of the meniscus but the rounded head of the tibia can move similarly within the concave undersurface. The limb mobility is increased by the virtual conversion of a single joint into two.

Another function of fibrocartilages, to act as thrust-pads, has been fully discussed by MacConaill (1932). This action will be important only where the articulating surfaces have large radii of curvature in cross-section and/or the thrust between them is very great. These conditions do not obtain in the neck of the pigeon or the superior tibio-fibular joints of the Tasmanian devil, but they are certainly present in the human knee joint where it is indeed likely that the menisci aid lubrication of the surfaces by acting as thrust pads.

Finally there are certain joints where the presence of fibrocartilages is difficult to explain. An example is the temporo-mandibular joint of carnivores, often stated to be a pure hinge-joint. Although this is not strictly accurate, for there is a small but functionally important lateral sliding of the head of the mandible in its socket during occlusion of the teeth (Sprinz, personal communication), it is unlikely that the existence of an intra-articular fibrocartilage here is related to this very slight movement. Study of the joint in the dog suggests an alternative explanation. Isolated forward or backward movement of each mandibular head in its socket cannot occur in carnivores. However, the head rolls forward or backward, and the thin biconcave disc slides within the temporal fossa, whenever the jaw is opened or closed. As a result, a particular area of the fossa is in contact with the mandibular head in each position of the lower jaw. This rolling movement of the head cannot occur unless the receiving surface is flatter than the entering surface: the presence of an intra-articular disc under muscular control will facilitate it by acting as a 'vehicle' for the moving mandibular head. Thus forward or backward rolling of the head will not only increase the range of movement at the temporo-mandibular joint but also diminish the risk of frictional attrition of the articular cartilage.

As discussed above, the stability of many joints depends upon as close a fit between the articular surfaces as is consistent with efficient lubrication. Intra-articular fibrocartilages allow this apposition, even in joints where the nature of the movements demands, in theory, marked incongruity between the entering and receiving surfaces. They are not universally found in such joints, however; stability may instead depend on a complex ligamentous mechanism as in the reptilian shoulder (Haines, 1952) or on powerful muscles attached close to the articular margin as in the jaw joints of certain marsupials (Parsons, 1900).

#### SUMMARY

1. Intra-articular fibrocartilages are reinforced by bony lunulae if they are steeply wedged in transverse section.

2. Fibrocartilages usually occur in those articulations where flexion and extension are associated with gliding, a combination of movements that demands a well-rounded entering surface and a relatively flattened receiving surface. The interposition of a fibrocartilage, the mobility of which is under ligamentous or muscular control, helps to prevent instability of the joint.



3. In bicondylar joints, gliding movement is usually necessitated by axial rotation. Where axial rotation occurs without gliding, as in the medial half of the knee of the tortoise, no fibrocartilage is present.

4. In some joints, notably the temporo-mandibular joint of carnivores, fibrocartilages may reduce the likelihood of attrition of the receiving articular surface by ensuring that a constantly changing region bears the pressure of the entering surface.

5. Fibrocartilages may also perform the functions of increasing the range of movement at a joint and acting as thrust-pads.

My thanks are due to Prof. D. V. Davies and Prof. M. A. MacConaill for criticism and advice, and to Mr G. Maxwell for technical assistance.

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# THE ELASTIC PROPERTIES OF THE ANTERIOR CRUCIATE LIGAMENT OF THE RABBIT

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## INTRODUCTION

In most current anatomical literature joint ligaments which do not contain a large proportion of elastic fibres are described as inelastic, and they are sometimes contrasted with structures such as nerve trunks and small arteries which are termed elastic. Clearly, in such descriptions, the term elasticity refers to that property of gross and reversible extensibility in response to small tensile stresses which is characteristic of thin rubber and weak springs. However, although this usage is common, the word elasticity is also used to denote a physical property which is only loosely related to the 'springiness' implied by its colloquial use, and it is in this technical sense that the word is used in this communication.

When any body is affected by a tensile stress, its length is altered, the extensibility varying in amount from substance to substance. If, when the stress ceases, the body returns to its original length it is elastic to a stress of that magnitude, whereas if it fails to do so it is inelastic or viscous. Each elastic body has an 'elastic limit': if the tensile stress is less than the elastic limit the body reacts elastically, whereas if it is greater the body fails to recover its original length when the stress ceases. Thus in determining whether a body is elastic or inelastic to a particular tensile stress, its complete return to its original length on release is the only relevant factor: the degree of temporary extension which the stress produces is of no significance. For example, steel and rubber are both elastic to tensile stresses of certain values. The elastic limit of steel is much greater than that of rubber, whereas the extension caused by a particular tensile stress is much greater in rubber than in steel.

This simple definition of elasticity takes no account of the duration of the tensile stress to which the body is subjected. Bodies which are elastic to a momentary stress of a certain magnitude may react in one of three different ways to a stress of the same magnitude which is maintained at a uniform value for some time.

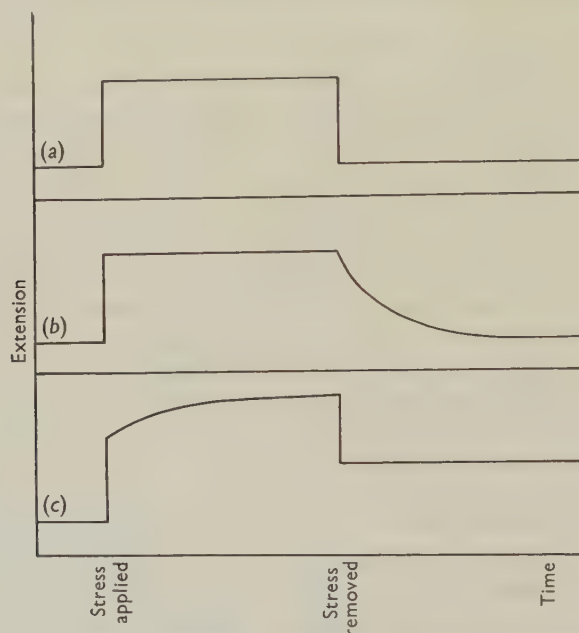
In the first type of reaction the body is extended when the tensile stress is applied, retains its new length while the stress is maintained, and regains its original length immediately the stress is removed. A body which reacts in this way is elastic to stresses of the magnitude and duration used in the experiment, and the reaction may be shown graphically, as in Text-fig. 1*a*.

The second type of reaction is similar to the first, except that when the tensile stress is removed, the original length is regained over a period of time, the duration of which is proportional to the duration of the preceding stress. A body reacting in this way is elastic, but shows the phenomenon of 'elastic after-effect' (Text-fig. 1*b*).

In the third reaction, the body is extended as tensile stress is applied to it, and the extension then gradually increases while the same stress is maintained. The body

may eventually rupture: on the other hand, if the stress is discontinued before rupture occurs, the body fails by a greater or lesser measure to regain its original length. A body reacting in this way is inelastic or viscous to stresses of the duration and magnitude used in the experiment (Text-fig. 1c).

Thus in the examination of the reaction of any body to a tensile stress, two dimensions of the stress—those of magnitude and duration—are both of fundamental importance.



Text-fig. 1. The reaction of elastic bodies to prolonged tensile stress.

#### THE LITERATURE

Annovazzi (1928), whose investigations were carried out on the knee joints of dogs immediately after death, found that the joint ligaments were appreciably extensible under load and that, within certain limits, they were also elastic, as they completely regained their original length when the load was removed. Furthermore, he demonstrated that the elastic limit (i.e. the maximum momentary load to which the ligament would react elastically) was less in ligaments containing elastic fibres than in those consisting entirely of collagen fibres. In keeping with Annovazzi's findings is the fact that joint ligaments which are both extensible and elastic are inherent in the concept of the 'Schnappgelenke' mechanism elaborated by Palmgren (1929) and Fick (1931), and discussed recently by Haines (1951). In contrast, Hardy (1951) came to the conclusion that the joint ligaments which he examined did not extend under load, but it is considered that his observations did not fully justify such a conclusion. The method which was used to determine the non-extensible nature of the cat's ligamentum patellae was not described, and furthermore, the fact that the greatest lengthening of the human spring ligament which was observed was not



mathematically significant, did not permit the assumption that no lengthening occurred.

Structures which are histologically similar to joint ligaments may be surmised to possess similar physical properties, and therefore the elastic properties of such structures are of some interest. Thus Wertheim (1847) and Hill (1951) have shown that the tendons of muscles are extensible and elastic. Gratz (1931), in his well-known study of human fascia lata, found that it was appreciably extensible and that it was elastic with a high elastic limit. And Dick (1951), working on human fascia lata and dura mater, observed 'that white collagenous fibres by themselves respond to distension as does rubber'.

It seems probable, therefore, that joint ligaments in common with other predominantly collagenous structures, extend when subjected to load, and that this extension is reversible within certain limits. However, information is incomplete, especially on the relationship of the reaction of a ligament to the duration of the load, and the present investigation was carried out mainly in an endeavour to elucidate this aspect of the problem.

#### MATERIAL

The investigation was carried out on the anterior cruciate ligaments of young rabbits of between 2 and 6 lb. body weight. This ligament was chosen for study, for the following reasons. Histologically it consists solely of collagen fibres and is devoid of elements showing the specific form and staining reaction of elastic fibres (Pl. 1, figs. 1, 2). The ligament is longer and stronger than most of the other ligaments of the rabbit, so that its use tends to reduce the percentage error in measurements of elongations and assessments of breaking loads. Furthermore, the ligament is attached centrally to the ends of the femur and tibia rather than marginally, a feature which assists considerably in the attachment of loads (*vide infra*), and lastly, it is discrete from the capsular ligament of the knee joint, so that the dissection required to isolate it leaves it undisturbed.

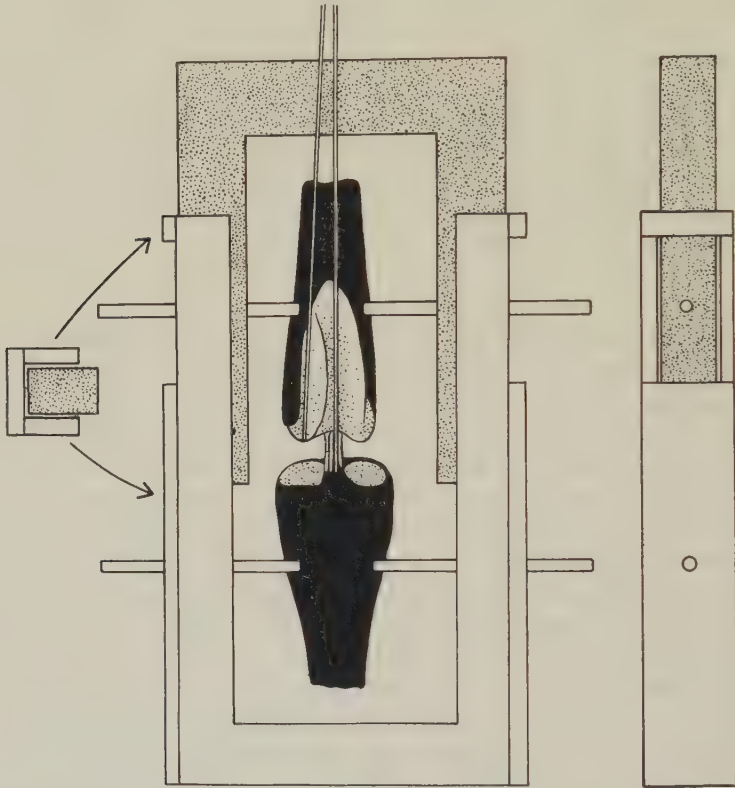
Annovazzi (1928) noted that the physical properties of ligaments change rapidly after general or local death. In my own experience ligaments become less readily extensible (Young's Modulus increases) and react elastically to greater loads (the elastic limit increases): the change becomes apparent within an hour of death and is progressive thereafter. The preparation of each ligament for examination was therefore always completed as quickly as possible, and no observations were considered valid unless they were made within 30 min. of death.

#### METHOD

In each experiment the animal was first weighed and then killed. The knee joint, with an inch or so of bone on either side, was immediately excised, and all connexions between the tibia and the femur except the anterior cruciate ligament were divided. A steel pin was then driven through the shaft of each bone from side to side, and the pins were attached to stirrups. The upper stirrup was fixed to a cross-bar, and to the lower one the necessary load was applied.

This method of loading a joint ligament has certain advantages over the method by which loads are attached to the isolated ligament by means of clamps, but it can

be used only when, as in this case, the ligament has central rather than marginal bony attachments. One advantage of the method is that there can be no slipping of the clamps, but an additional and more important advantage is the fact that the whole of the ligament, including its attachment to bone, is examined. Ham (1953) has observed that the histological appearance of the intermediate part of a ligament differs from that of the same ligament at or close to its attachment. Near the attachment the fibroblasts become rounded and encapsulated and there is a considerable



Text-fig. 2. The interlocking stirrups. The stirrups with a knee-joint ready for examination are shown in the centre. On the right is a side view, and on the left a cross-section through the parts indicated.

increase in the amount of amorphous intercellular substance. The difference is shown in Pl. 1, figs. 3 and 4. The possibility has to be recognized, therefore, that the elastic properties of different parts of a ligament may vary; for this reason examination of part of a ligament gives no certain assessment of the properties of the whole structure.

As the anterior cruciate ligament passes from the tibia to the femur, it twists through  $90^\circ$ , as if the femur had been rotated medially to that extent upon the tibia. For this reason, when all other connexions between the tibia and femur are divided, tension on the ligament tends to undo this twist with a consequent elongation of the ligament which is not due to extension of its fibres. To obtain a correct assessment

of the actual lengthening of the ligament under load, rotation of one bone on the other must be prevented. This was done by interlocking the two stirrups as shown in Text-fig. 2, so that although they could approach or recede they could not twist or tilt in relation to one another. Although some friction must occur between the two stirrups it was considered that because of the relatively large loads used in the investigation, it could be safely discounted.

#### OBSERVATIONS

*The relation of the breaking load of the anterior cruciate ligament to the weight of the rabbit*

The breaking load of a ligament is that which causes rupture of the ligament as soon as it is applied. In the case of the anterior cruciate ligament of the rabbit the rupture constantly occurs at its attachment to the tibia, and a flake of bone is always torn away.

The breaking loads of twenty-seven ligaments have been examined by the method described below. Each specimen was fitted into interlocked stirrups and a counterpoised metal drum was suspended from the lower stirrup. The drum was rapidly filled with water from the tap until the ligament ruptured, when the tap was immediately turned off. The drum, still counterpoised, was weighed to the nearest quarter of a pound by means of a spring balance, and this weight was taken as the breaking load of the ligament.

The results which were obtained are shown in Text-fig. 3, in which the breaking load of the anterior cruciate ligament is plotted against the body weight of the animal. Although there is considerable individual variation, the breaking load of the anterior cruciate ligament is approximately proportional to the cube of the body weight. The upper dotted line in Text-fig. 3 expresses the equation:

$$3.4 \text{ (breaking load)} = (\text{body weight})^3.$$

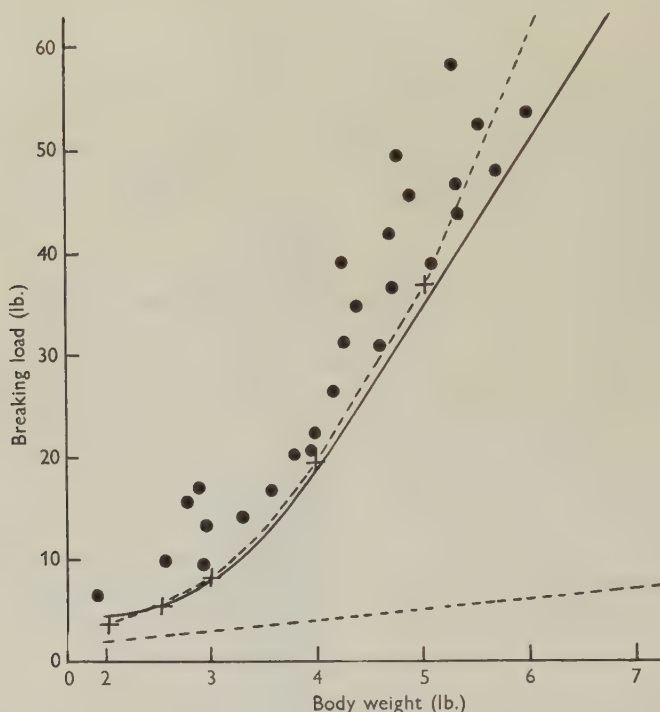
The relationship is not sufficiently uniform to permit the calculation of the breaking load of a given ligament, but on the other hand, it makes it possible to estimate the greatest load which can be applied to the ligament of a rabbit of known weight without risking its rupture. Such a load will be referred to subsequently as the submaximal load and it is indicated by the continuous line in Text-fig. 3.

*The effect of load on the length of the ligament*

Each specimen was prepared and fitted into the interlocking stirrups as described above. When a load is applied through the lower stirrup to the tibia, the tibia moves downwards but because the upper stirrup and pin and the other apparatus fixing the femur are themselves distorted by load, the femur will also move downwards. Thus the actual change in length of the ligament due to a load is equal to the difference in the downward displacements of the tibia and femur. For this reason cotton threads were attached to both the tibia and the femur as closely as possible to the areas of attachment of the ligament to those bones, and the threads were led off and attached to two magnifying arms which drew tracings on a smoked drum. In all the kymographs shown here the upper tracing represents the femoral attach-



ment of the ligament, and the lower tracing the tibial attachment. Vertical displacement of either end of the ligament is indicated by a proportionate deviation of the corresponding tracing in the opposite direction. Thus when a load is applied to a ligament both tracings ascend and any elongation of the ligament is shown by a proportionate decrease in the distance between the two tracings: when a load is removed, both tracings descend and any contraction of the ligament is indicated by



Text-fig. 3. The relationship of the weight of the animal to the breaking load of the anterior cruciate ligament. The upper dotted line represents the equation  $3.4 \text{ (breaking load)} = (\text{body weight})^3$ ; the continuous line indicates the submaximal loads, and the lower dotted line denotes loads equal to the body weight.

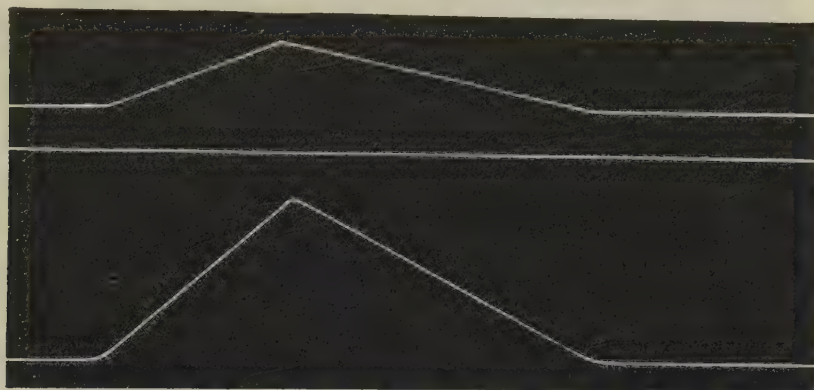
proportionate increase in the distance between them. The magnifying arms were so arranged that the actual change in the length of the ligament was magnified more than ten times. The distance between the tracings was measured by calipers which were accurate to  $\frac{1}{100}$  in., so that a change in the length of the ligament was measured correct to  $\frac{1}{1000}$  in. In the several kymographs shown in this paper, the initial distance between the tracings and the speed of the tracings both varied, and in the final reproduction the reduction differs in each case.

### Experiment 1

Six ligaments were subjected to loads which in each case increased at a rapid and uniform rate to the appropriate submaximal value indicated in the graph in Text-fig. 3 and then similarly decreased to zero. The load was increased by filling the

counterpoised drum already mentioned with water from the tap, and decreased by removing the water from the drum by a suction pump.

In the experiment illustrated in Text-fig. 4 the ligament was taken from a rabbit of 5 lb. body weight. The two tracings approach one another as the load is applied, indicating a proportionate elongation of the ligament, and return to their original relationship as the load is removed, indicating the recoil of the ligament to its original length. Thus it is apparent that under these circumstances, the ligament is both extensible and elastic.



Text-fig. 4. Rapid loading and unloading of the anterior cruciate ligament.

The tracings produced while the ligament was loaded and unloaded are straight lines, and because the load was applied and removed at uniform rates, it follows that the extension of the ligament was always proportional to the load. The response of the ligament thus conforms to Hooke's Law for elastic bodies, i.e. strain =  $C \times$  stress, where  $C$  is the modulus of elasticity.

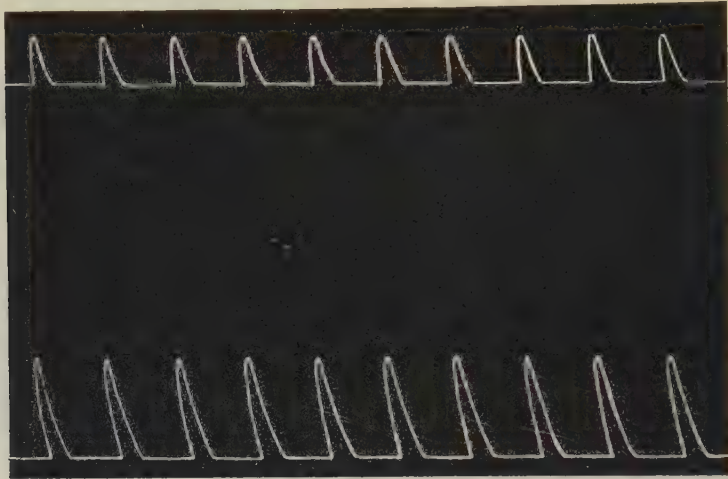
The actual extension of the ligament in the experiment illustrated in Text-fig. 4 was  $\frac{8.2}{1000}$  in., and the approximate average length of the fibres of the ligament was about  $\frac{1}{3}$  in. These measurements indicate that the ligament was temporarily extended by the submaximal load more than 20% of its original length. The extension of ligaments by full submaximal loads varied from specimen to specimen, but it was always easily appreciable even by naked eye examination. Pl. 1, fig. 5, is a photograph of a specimen fixed in the extension apparatus, and the area outlined by the dotted lines indicates the area shown in the three photographs in Pl. 1, figs. 6-8. In fig. 6, the load on the ligament was 2 lb., in fig. 7, it was 25 lb., and in fig. 8, it was 48 lb.; the progressive extension of the ligament, as measured by the separation of the stump of the posterior cruciate ligament from the lateral femoral condyle, is readily apparent.

### *Experiment 2*

Six ligaments were subjected to repeated submaximal loads at 1 min. intervals, the load being applied and then immediately removed on each occasion. Text-fig. 5 shows the tracings from such an experiment when a load of 42 lb. was applied

10 times to the ligament of a  $5\frac{1}{2}$  lb. rabbit. The two tracings approach each time the load is applied, but return to their original relationship each time the load is removed. This indicates that the ligament lengthens with each application of load but retains its original length at the end of the experiment.

The anterior cruciate ligament is therefore elastic to a submaximal load of short duration, even if this load is repeated several times at short intervals.



Text-fig. 5. The effect on the anterior cruciate ligament of intermittent submaximal loads of momentary duration.

#### *Experiment 3*

Six ligaments were subjected to loads equal to the body weight of the animal from which they were obtained, for a long period arbitrarily fixed at 5 min. A typical experiment is illustrated by Text-fig. 6. The tracings separate as the load is applied, remain parallel while the load is constant, and return to their original relationship as soon as the load is removed. The form of the tracings indicates that the ligament extends as the load is applied, remains of uniform length while the load remains constant and returns to its original length when the load is removed.

Thus the anterior cruciate ligament is elastic to a load of the order of body weight, even if this load is maintained for a considerable time.

#### *Experiment 4*

Six ligaments were subjected to submaximal loads for a period of 5 min. It was found that the ligament extended rapidly as the load was applied, and then continued to extend more gradually while the load was maintained. In some of the experiments the ligament ruptured before the completion of the period of 5 min., and such a case is illustrated by the tracings shown in Text-fig. 7*a*. The initial approach of the tracings is due to the elongation of the ligament on application of the load. The subsequent more gradual approach of the tracings indicates a progressive extension of the ligament occurring while the load was constant at the submaximal value.



In other cases the ligament was still intact at the end of 5 min., and such an experiment is illustrated in Text-fig. 7*b*. The ligament extended as the load was applied and then continued to extend while the submaximal load persisted, as indicated by the gradual progressive approach of the two tracings. When the load was removed the tracings separated but did not achieve their original relationship. The permanent reduction in the distance between the two tracings signifies a proportional permanent elongation of the ligament. Subsequent histological examination of the ligament in this experiment showed no rupture of the fibres, and it is to



Text-fig. 6. The effect on the anterior cruciate ligament of a prolonged load equal to the body weight of the animal.

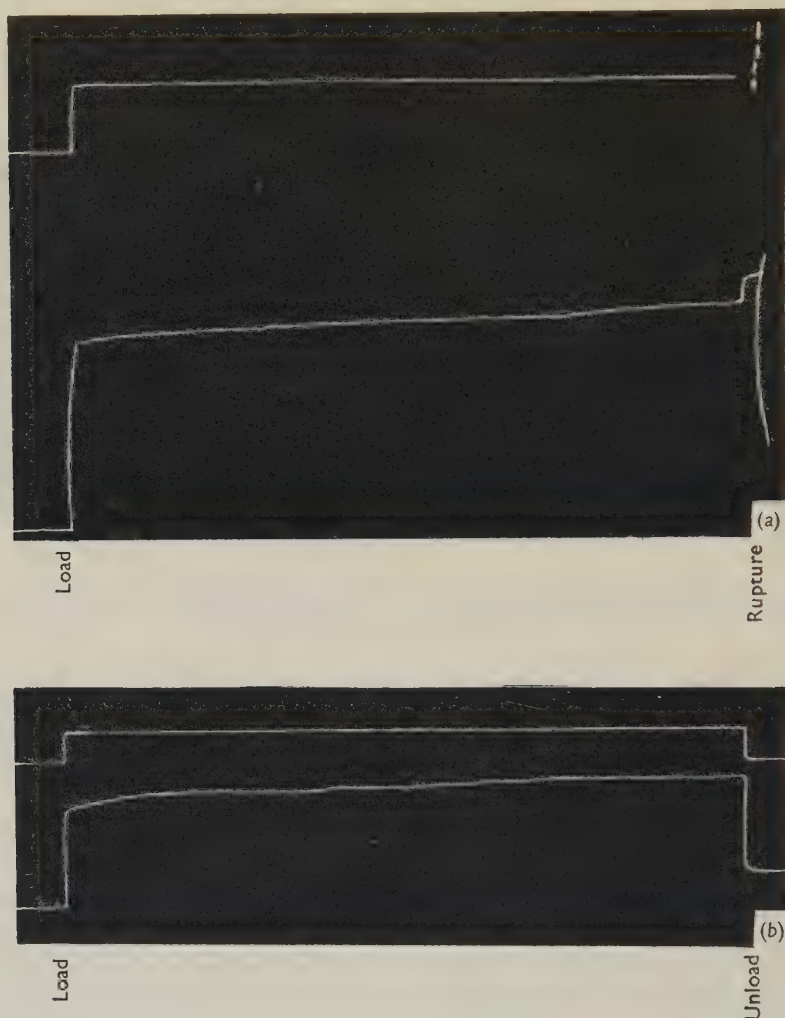
be presumed that the permanent elongation is related to the fibres themselves. Thus the anterior cruciate ligament is not elastic to a submaximal load of long duration, but acts, in those circumstances, as a viscous body.

#### DISCUSSION

The observations which have been made during this investigation show that the anterior cruciate ligament of the rabbit is an appreciably extensible structure. Furthermore, the ligament is elastic to loads of the order of body weight, and this property is independent of the duration of the load within the limits likely to be experienced during life. The ligament is also elastic to what has been described as submaximal loads, either single or repeated, provided that each load is of a very short duration. A load may be outside the elastic limits of the ligament either because of its magnitude alone, or because of the combination of its magnitude and its duration. In the former case, the load is equal to or greater than the breaking load and the ligament ruptures as soon as it is applied. In the latter case, the load is less than the breaking load, but if it is prolonged, the ligament will either rupture after an appreciable interval or will remain elongated after the load has been removed.

The magnitude and duration of the stresses which affect the joint ligaments of

a living experimental animal, vary with the posture and activity of the animal. When the animal is stationary, the stresses are usually of small magnitude, but may persist continuously for some time. The average duration of stress would appear to vary in different animals and in different parts of the same animal, but it is usually



Text-fig. 7. The effect of prolonged submaximal loading of the anterior cruciate ligament. *a*, rupture of the ligament after 4 min.; *b*, load removed after 5 min.

of the order of 1 min.; in the case of the trunk and lower limbs of man it has been shown (Smith, 1953) that the average duration of immobility during standing is 30 sec. It has been demonstrated that the anterior cruciate ligament of the rabbit is elastic to a load equal to body weight, maintained for as long as 5 min., and it is therefore suggested that this ligament is elastic to the stresses which may affect it when the animal is stationary.

During movement, it is doubtful whether joint ligaments are subjected to any considerable stress, as long as progress continues on a smooth surface, and in an intended direction, because in those circumstances, the joint is under full muscular control. Nevertheless, unexpected joint displacements frequently occur during movement, and if they take place with a force or a speed too great for the defensive muscular mechanism, the joint ligaments are subject to a stress which may be very large, but which persists for a very short time. The stress may be sufficiently large to rupture the ligament at once, but if rupture does not occur, the observations recorded in Exp. 2 would indicate that the joint ligament will react elastically even if the stress is repeated several times.

#### SUMMARY

1. The elastic properties of the anterior cruciate ligament of the rabbit have been studied within 30 min. of the death of the animal.
2. The breaking load of the ligament is related to the weight of the animal.
3. The ligament can be temporarily extended by as much as 20 % of its original length.
4. The ligament is elastic to a load of the order of body weight maintained for 5 min.
5. It is elastic to submaximal loads of short duration.
6. It reacts as a viscous body to submaximal loads of long duration.

My thanks are due to Prof. R. Walmsley for his interest and advice. I am also indebted to Mr J. Brown who prepared the photographs and photomicrographs in the plate.

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\* Original not available. Abstract only consulted.



## EXPLANATION OF PLATE

- Fig. 1. The anterior cruciate ligament of the rabbit. Weigert's elastin stain. ( $\times 250$ .)
- Fig. 2. One of the genicular arteries. Section from same block as that in fig. 1. Weigert's elastin stain. ( $\times 250$ ).
- Fig. 3. The intermediate part of the anterior cruciate ligament of the rabbit. Haematoxylin and eosin. ( $\times 250$ ).
- Fig. 4. The attachment of the anterior cruciate ligament of the rabbit to the tibia. Ligament above, bone below. Haematoxylin and eosin. ( $\times 250$ .)
- Fig. 5. Rabbit's knee joint with all connexions between the femur and tibia divided except the anterior cruciate ligament. The area enclosed by the dotted line is that shown in figs. 6-8. ( $\times 1.3$ .)
- Fig. 6. The anterior cruciate ligament under a load of 2 lb. ( $\times 3$ .)
- Fig. 7. The same ligament under a load of 25 lb. ( $\times 3$ .)
- Fig. 8. The same ligament under a load of 48 lb. ( $\times 3$ .)



ITH—ELASTIC PROPERTIES OF ANTERIOR CRUCIATE LIGAMENT OF THE RABBIT





# THE DEVELOPMENT OF THE VENTRICULAR PART OF THE CONDUCTING TISSUE IN THE HEART OF THE SHEEP\*

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## INTRODUCTION

The mode of development of the atrioventricular bundle has been interpreted in several ways. A process of growth into the ventricles from a supraventricular structure is supported by one group of workers, whereas others consider it developing *in situ* from the ventricular myocardium. The suggestion that the Purkinje fibres are embryonic cardiac muscle, which has not pursued its normal development, is closely associated with the latter group.

The development of the conducting tissue has been studied in sheep, pig, rabbit, calf and man; and it is possible that the great variation in the adult structure of the conducting system in these mammals may have given rise to the different opinions regarding its origin. This suggestion is supported by the fact that there is considerable agreement between the observations of the two previous workers on the sheep, Sanabria (1936) and Field (1951*a*). They both describe the development of the common trunk of the bundle from the musculature of the dorsal wall of the atrioventricular canal, and they agree that the Purkinje network is differentiated from the ventricular myocardium.

Although the general observations of Sanabria and Field are in agreement, the former describes an orderly spread of differentiation into the ventricles from the common trunk through the main branches to the peripheral network; while the latter sees the Purkinje tissue arising as fibres arrested in development because they are not concerned with the work of active contraction.

Davies & Francis (1941, 1946, 1952) and Davies (1942) regard the conducting tissue as a neomorphic development, and a specialization for a particular purpose in homoiothermal vertebrates. These authors (1946) accept the opinion that the conducting tissue arises as a new growth into the ventricles as support for their theory; in 1952 they indicate their belief that proof of the differentiation of Purkinje fibres from primitive mesenchymal cells along their own line of development may be regarded as support for the same view.

## MATERIAL AND METHODS

All the material used was obtained from the Edinburgh abattoir. The young embryos were fixed in Bouin's fluid while the foetal and adult specimens were fixed in 10% formalin or in Susa, the latter being the fixative of choice.

\* Based on part of a thesis accepted for the M.D. degree of the University of Edinburgh in July 1952.

The following material was examined:

- (i) Complete serial sections of eighteen embryos from 5.4 to 18.4 mm. c.r. length.
- (ii) Complete serial sections of the thorax of nine embryos from 20.1 to 44 mm. c.r. length.
- (iii) Complete serial sections of the heart of eight embryos from 63 to 210 mm. c.r. length.
- (iv) Serial sections from selected blocks of the heart from twelve embryos from 250 to 400 mm. in length. [A sheep embryo 400 mm. in length is covered with hair, possesses hoofs and is near term.]
- (v) Serial and interrupted sections from selected blocks from six adult sheep hearts.

Hollande's chlorcarmine stain, as described by Lee (1937), was found to give good cytological detail in both the embryonic and adult myocardium, and this stain has been chiefly employed. Although Rossman's alcoholic fixative gave the best results when using Bauer's method (Lee, 1937) for glycogen, satisfactory results were obtained with Susa fixation. Adult and late foetal Purkinje tissue was clearly demonstrated by Bauer's method followed by Hollande's stain.

#### OBSERVATIONS

The cytological changes which characterize the conducting tissue of the early embryo can be recognized with greater certainty if they are compared with the well-defined structures of late foetal life. During this investigation, therefore, the later stages were examined first, but the description will be given in the natural chronological order.

##### *Atrioventricular node and bundle*

As soon as the muscular interventricular septum is developed (6-7 mm.) a change in structure can be seen in the dorsal wall of the atrial canal, immediately cephalic to the dorsal end of the interventricular septum, as it passes behind the dorsal endocardial cushion. In this situation the myocardial fibres have well-marked cell membranes, transparent cytoplasm and dark, ovoid nuclei. Although these myocardial fibres have lateral connexions, their long axes are directed along the free edge of the septum (Pl. 1, fig. 1). This orientation is in contrast to the muscular arrangement in 5-6 mm. embryos where the fibres are arranged circularly around the atrial canal.

These pale cellular strands can be traced up to the caudal end of the interatrial septum, at the point where the right venous valve is attached in relation to the left horn of the sinus venosus. The atrial myocardium is continuous with these strands without the intervention of any specialized structure (Pl. 1, fig. 1). At their ventricular end the modified strands are continuous with the fibres of the interventricular septum (Pl. 1, fig. 2). This group of pale strands on the crest of the muscular interventricular septum has been recognized as the first sign of the common trunk of the atrioventricular bundle.

Although at first there is no evidence of a node at the atrial end of the bundle, by the 11-12 mm. stage the cells at its upper end can be seen to be smaller and more darkly stained than the elements of the bundle itself. The cells of the node are closely packed so as to form a readily defined mass. There is, from its inception, complete

continuity between the atrial myocardium and the cells of the atrioventricular node (Pl. 1, fig. 2); this state is not altered throughout development or in the adult (Pl. 4, fig. 16), in spite of the statement by Glomset & Glomset (1940) that they could not find any connexion between the node and the atrial muscle in ungulates.

The further development of the atrioventricular node will not be included in this paper, but some observations which have a bearing on the development of the ventricular system must be mentioned. The nodal cells do not appear to be actively dividing at any stage of development and no mitotic figure has been seen amongst them. The node is relatively reduced in volume with increasing age (Table 1). In spite of the difficulties and consequent inaccuracies in the estimation of the weight and volume, a tenfold reduction in the ratio is regarded as significant.

Table 1

Length of embryo (mm.)	Weight of heart (g.)	Volume of node (mm. <sup>3</sup> )	Ratio: Volume of node Weight of heart
12	0.021	0.0028	0.13
23	0.038	0.0048	0.16
45	0.152	0.013	0.085
95	0.825	0.07	0.085
149	2.85	0.085	0.03
210	5.85	0.24	0.01

These figures should be regarded as only approximate. The volume of the node is expressed as the product of its greatest dimensions. The hearts were weighed after they had been removed from embryos whose length corresponded with a specimen which had been serially sectioned.

*Later development of the common trunk of the atrioventricular bundle*

The pre-natal changes in the shape of the common trunk are those of lengthening and relative attenuation (Pl. 1, fig. 2; Pl. 3, fig. 12; Pl. 4, fig. 16). The bundle at 11.7 mm. is 350 $\mu$  in length and the diameter is 100 $\mu$ , whereas the common trunk of the 210 mm. foetus is 2500 $\mu$  long and the diameter has increased to only 200 $\mu$ . Changes in the general structure of the developing heart are responsible for these alterations in the shape and relations of the bundle (see Text-fig. 1).

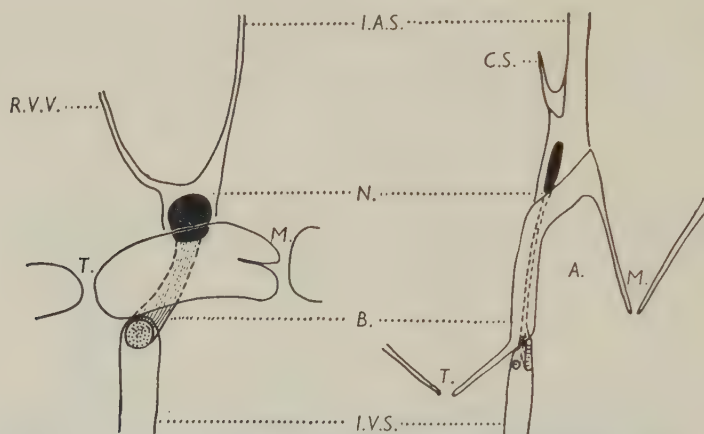
The lateral connexions of the fibres of the bundle, which can be seen in the early embryo, become less prominent and by 20 mm. the common trunk is a cylindrical fascicle of pale fibres with a few striated myofibrils (Pl. 2, figs. 6, 7). The dark ovoid nuclei of these fibres, compared with the vesicular nuclei of the surrounding myocardium, are a characteristic feature. The transverse diameter of the fibres is not significantly greater than that of the ventricular muscle fibres until the 30–40 mm. stage (Pl. 3, fig. 14). But in the later stages the modified fibres, which can now be called Purkinje fibres, increase in size until their diameter is 60 $\mu$  while the ordinary myocardial fibres remain with a diameter of 10–12 $\mu$ . The cytoplasm of the Purkinje fibre of the common trunk is pale until the 100 mm. stage, but from 100 to 300 mm. it is dark and granular. It was possible to demonstrate glycogen at 200 mm., and it may be suggested that these granules are related to glycogen metabolism. The cytoplasm of the mature Purkinje fibre is pale and shows a strong reaction with Bauer's reagent. Double nuclei are not a prominent feature of the fibres in the common trunk until 300 mm.

The common trunk in the sheep does not give any branches to the surrounding



myocardium; from the 20 mm. stage onwards it is progressively isolated by its developing fibrous tissue sheath (Pl. 3, fig. 14).

While it is possible to make the statement that the common trunk of the bundle is differentiated before the peripheral atrioventricular muscular connexions are severed by the invasion of connective tissue, it is difficult to define the stage when the bundle is left as the only muscular connexion between the atria and the



Text-fig. 1. Diagram showing the relations between the atrioventricular bundle and the pars membranacea septi in the early embryo (left) and in the adult (right). *N.* and *B.*, atrioventricular node and common trunk of the bundle; *I.A.S.* and *I.V.S.*, interatrial and interventricular septa; *M.* and *T.*, mitral and tricuspid orifices; *C.S.*, coronary sinus; *R.V.V.*, right venous valve; *A.*, aortic vestibule.

ventricles. By the 30 mm. stage a complete collagenous ring can be demonstrated histologically, but it is probable that the muscular connexions are broken by 15 mm. Field (1951*a*) points out the difficulty in assessing the stage when the peripheral connexions are severed, but he is inclined to agree that the bundle can be identified before the atria and ventricles are completely separated by fibrous tissue.

#### *The left branch of the atrioventricular bundle*

In a 13 mm. specimen the ventricular end of the common trunk is continuous with the interventricular septum, and there is no evidence of bifurcation. But in later stages the differentiated cells, similar to those of the common trunk, can be traced into the spongy myocardium on the left side of the interventricular septum. In an 18.7 mm. specimen the point of transition between these paler cells and the darker, normal muscle cells can be seen on the left side of the septum. Individual strands of fibres can be traced without a break in continuity through a gradual transition (Pl. 1, figs. 3–5). By the 35 mm. stage the differentiation can be traced down to the small fasciculi which traverse the left ventricle near its apex, and transitions can be found at the bases of the papillary muscles.

The left branch of the bundle is originally differentiated in the most superficial part of the spongy myocardium, but it is subsequently absorbed from above down-

wards into the subendocardial tissue of the compact interventricular septum. Many strands can be seen to leave the left branch and pass into the septum where they are continuous with the muscle of the septum.

The developmental changes in the cytology of the left branch are the same as those described in the common trunk of the bundle. The constituent fibre of the adult left branch is the typical Purkinje fibre.

#### *The right branch of the atrioventricular bundle*

The differentiation of the right branch occurs in the same general way as has been described for the left. The differentiation can be traced farther into the ventricles with increasing age, and progressive partial absorption of the branch into the septum occurs in the same manner. The lower part of the right branch which is not absorbed into the septum becomes the conducting tissue portion of the moderator band. Field (1951*a*), in his fig. 23, draws attention to the absorption of the right branch, but he does not refer to the same phenomenon in the left branch.

The compact right branch, which does not give any branches until it reaches the free wall of the right ventricle, allows the cytological changes to be studied more easily than in the more diffuse left branch. At 20 mm. the region of transition is where the right branch leaves the common trunk; and it was observed that the change is more abrupt in the right branch than in the left, and a discrete fascicle of normal muscle extending downwards from the transition could be traced for a short distance in a subendocardial position before it passed out into the moderator band. This re-orientation of the myocardium below the elements which have become conducting tissue, to form a pathway along which the differentiation can pass still farther (Pl. 2, figs. 6–11), seems not to have been noticed previously. The differentiated right branch can be traced into the moderator band by 45 mm. and to the free wall of the ventricle by 60 mm.

From these descriptions it appears that the right branch is developed considerably later than the left branch, as has been noted by Mall (1912) and Walls (1947).

A definite fascial sheath could be recognized along the whole length of the right branch by 100 mm.

#### *Subendocardial Purkinje network*

Fibres which are larger and less clearly striated than the normal myocardium and which possess more spherical nuclei can be recognized under the endocardium at 70 mm.; at this phase of development these fibres are only found around the papillary muscles in the left ventricle and close to the attachment of the moderator band in the right ventricle. It is possible to be certain that these fibres will become the mature Purkinje fibres because those in the right ventricle can be traced, on the one hand, into continuity with the right branch of the bundle in the moderator band, and through a gradual transition to normal myocardium on the other. These earliest Purkinje fibres do not enter the walls of the ventricles (Pl. 3, fig. 15).

As development proceeds these Purkinje fibres become progressively larger and paler than the ordinary cardiac muscle-fibres. Double nuclei appear at 100–150 mm. and at about the same time these fibres could be clearly demonstrated by their glycogen content.

*Intramyocardial Purkinje network*

The differentiated Purkinje fibre cannot be traced into the walls of the ventricles until after 100 mm. But in all the later stages there is an intramural network which is as profuse as the subendocardial network. This has been pointed out in the ox by Abramson & Margolin (1936). In these later stages, frequent abrupt transitions can be traced between the Purkinje fibres and the normal myocardium (Pl. 4, fig. 17). Although in serial sections apparent continuity is often seen it is rare to find a junction cut in such a fortunate plane as in Pl. 4, figs. 18, 19. In such a transition the continuity between the myofibrils of the Purkinje fibre and those of the myocardium is evident, the glycogen content of the fibre abruptly diminishes at the site of transition and the fibrous tissue sheath of the Purkinje fibre becomes continuous with the interstitial connective tissue of the heart muscle. The continuity between Purkinje fibre and normal myocardium is contrary to the statement of Glomset & Glomset (1940) that the terminal ramifications of the Purkinje network are always separated from the heart muscle by connective tissue.

Cardwell & Abramson (1931), in a description of the Purkinje network in the ox, assert that the right and left Purkinje networks are continuous with each other through the interventricular septum. But the injection method of study, which they used, is subject to the criticism that the injected material can pass beyond the point of transition to normal myocardium and so give an erroneous impression of specialized continuity between the two systems. In serial sections of the interventricular septum from a 300 mm. heart, well-differentiated strands of Purkinje fibres could be traced into the septum from both subendocardial plexuses, more from the left than from the right. But these fibres always ended by transition to the normal myocardium and it was never possible to trace a specialized continuity through the septum.

*Cell division in the Purkinje fibres*

A mitotic division has not been observed in a cell which belonged to the conducting tissue of the sheep. This negative feature was particularly notable in the younger specimens when mitosis was frequently observed in the surrounding myocardium. The problem of amitotic division is suggested by the presence of double nuclei, but it can be stated that no definite evidence of this type of division has been seen. Superimposition of nuclei gave rise to false illusions of 'dumb-bell' and 'hour-glass' nuclei.

*The nervous elements of the atrioventricular tissue*

Specific stains for nervous tissue have not been employed in this investigation. Nerve ganglia were seen in relation to the atrioventricular node at 40 mm., and the presence of nerve trunks amongst the muscle fibres of the common trunk of the bundle was noted at 100 mm. (Pl. 3, figs. 12, 13). In the later stages the nerve trunks play a prominent part in the formation of the common trunk and its main branches. Nerve cells were not seen in relation to the Purkinje tissue of the ventricles. The presence and distribution of the nervous tissue of the ventricles has been recently reviewed by Davies, Francis & King (1952). These authors state that in *Artiodactyla* there are numbers of nerve cells in relation to the bundle and its main branches.



#### DISCUSSION

The published accounts, by Sanabria (1936) and Field (1951*a*), on the early development of the atrioventricular node and bundle in the sheep, agree with each other and with the present observations. Sanabria recognized the common trunk at 5 mm., but he used the re-orientation of the cells of the atrial canal as his criterion, and, according to him, the node itself is not apparent until 20 mm. Field noticed the re-orientation at 4.5 mm., but he did not observe cellular differentiation in the common trunk until 10 mm., or the appearance of a nodal structure until 14 mm. But the later development of the ventricular part of the conducting tissue has been interpreted in several ways, and these accounts may be arranged into three groups.

(*a*) Retzer (1908), Tandler (1912), Shaner (1929) and Walls (1947) consider that the bundle and its branches grow down into the ventricles as a new growth from a supraventricular structure. Shaner and Walls describe the growth from the node, and the former infers that the bundle proceeds into the ventricle to insinuate itself amongst the ventricular myocardium. The observation in this work of continuity between the distal end of the bundle and the ventricular myocardium at all stages of development is at variance with Shaner's opinion. Walls bases his view on his observation that the node appears to be a region of active growth, but he does not describe mitosis in the node and thus relies on its increased nuclear density. Mitotic figures were not seen in the conducting tissue in this investigation and this confirms previous work on the sheep by Sanabria (1936) and Field (1951*a*). While the latter considers amitotic division probable, the former agrees with the present author when he states that he was not able to see the least trace of amitotic division. It must be noted, however, that Duckworth (1952), working on human material, illustrates a mitotic figure in the atrioventricular node at 130 mm. As Sanabria, Field and the present author have noted that the common trunk of the bundle appears before the node in the sheep, it seems to follow that the bundle in this animal does not arise from division in the nodal tissue; and the agreed absence of mitosis in the bundle itself appears to exclude the possibility of its interstitial growth.

(*b*) Ranvier (1882), and other early workers, suggest that the Purkinje fibres are embryonic cardiac muscle fibres remaining amongst fibres which have pursued their development. This view is supported by Field (1951*a*), who makes the suggestion that these arrested fibres do not remain entirely unchanged while development proceeds and so reconciles the fact that the adult Purkinje fibre only slightly resembles embryonic cardiac muscle. Field describes the sporadic appearance of islands of Purkinje fibres in the subendocardium as early as 18.5 mm., but the present author could not see Purkinje fibres in that situation until the differentiation of the main branches can be traced into the ventricles, that is around 70 mm. As the fibres of the ventricular wall are all similar until the 60 mm. stage, it may be concluded that the Purkinje fibre appears as a modification of the myocardial fibre which has so far proceeded along a normal developmental line. The constant, orderly development of the conducting tissue to produce a complete, integrated system can be cited as additional evidence against Field's suggestion (1951*a*) that the Purkinje fibres acquire their adult structure because they lie in places of quiescence and are so arrested in their development.

(c) Mall (1912), Waterston (1918), Steinon (1925) and Sanabria (1936) describe the development of the Purkinje fibre *in situ* from the ventricular myocardium. Mall and Waterston do not describe the cytological development of the bundle, and their accounts of the development of the human heart would indicate that they regard the bundle as a remnant of the original complete atrioventricular continuity. While the present observations agree that the common trunk appears as a differentiation of the wall of the atrial canal, the profound changes in its structure, even before the peripheral atrioventricular muscular connexions are destroyed, indicate that the bundle is a new development for a particular purpose and not an ontogenetic remnant. Davies & Francis (1941), mainly on phylogenetic grounds, reached the conclusion that the bundle was a neomorphic development and not a remnant of the complete atrioventricular continuity of lower animals.

The observation that the differentiated cells can be traced farther into the ventricles with each succeeding stage of development is entirely opposed to the account of Steinon (1925) who describes the formation of the common trunk by an upward migration of Purkinje fibres which were differentiated *in situ*.

Sanabria (1936) describes the development of the branches of the bundle 'par un phénomène d'induction, ... émanant des éléments spécialisés du tronc commun...' and this view is supported by the present observations. Sanabria does, however, state that the branches of the bundle are formed from trabeculae of the spongy myocardium and that they are secondarily fused with the common trunk before they are differentiated; on this point the author would disagree with Sanabria and consider that the branches are formed by a continuous spread of differentiation into the spongy myocardium.

The re-orientation of myocardial fibres below the region of transition from modified myocardium to form a pathway along which the differentiated elements can be traced later in development was observed in this work. This is regarded as strong evidence that the modified fibres are formed from the ventricular muscle and is also in opposition to any concept of growth within the bundle itself.

Cohn & Trendelenburg (1910), Glomset & Glomset (1940) and Field (1951*b*) have stressed the difficulty in assessing the functional importance of the conducting tissue because direct physiological experiments interfere with the nerve trunks as well as with the differentiated muscle bundles. But effective indirect evidence in favour of muscular conduction has been supplied by Davies & Francis (1952), who showed a relationship between the size of the heart, the length of the QRS interval and the diameter of the Purkinje fibres in a large series of mammals and birds.

This embryological study furnishes several points which have a bearing on this functional question. The differentiation of the Purkinje fibres from myocardial fibres far advanced along the normal mode of development does seem to indicate an active specialization for a definite function; the differentiation would otherwise seem without purpose. The development of the left branch before the right may be related to the subsequent greater functional importance of the left ventricle. The absence of mitosis in the Purkinje fibre may be indicative also of a specialized structure, and this conclusion is supported by the demonstration by Erlanger (1909) that the bundle does not regenerate after injury.

While it is admitted that any conclusion regarding function which is based on

a developmental study must be regarded as tenuous, it may be stated with some assurance that the developmental history of the Purkinje fibre does suggest that it is a specialized structure for a particular function.

#### SUMMARY

1. The development of the atrioventricular bundle and its branches has been studied in forty-seven pre-natal and six adult sheep's hearts.

2. The common trunk of the bundle is the first portion of the conducting tissue to appear, at 7 mm., as a differentiation of the dorsal wall of the atrial canal. The atrioventricular node can be recognized at 11 mm. and at all stages of development its fibres are continuous with both the atrial muscle and the bundle.

3. The main branches and the terminal network are formed by a ventricular spread of differentiation from the fibres of the common trunk. The left branch is evident before the right, the subendocardial network could be seen at 70 mm. but the intramyocardial fibres could not be seen until 100 mm.

4. Mesenchymal cells which are to become Purkinje fibres, follow the normal development of myocardium until a later differentiation produces the Purkinje fibre.

5. There is continuity between the ventricular ends of the differentiated fibres of the conducting tissue and the normal myocardium throughout development.

6. The significance of these findings in relation to the morphology of the conducting tissue is discussed.

I wish to express my gratitude to Prof. J. C. Brash for suggesting this research, providing all facilities and assisting in the preparation of this paper. I would also like to thank Drs G. J. Romanes and J. W. A. Duckworth for their continued interest and encouragement.

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#### EXPLANATION OF PLATES

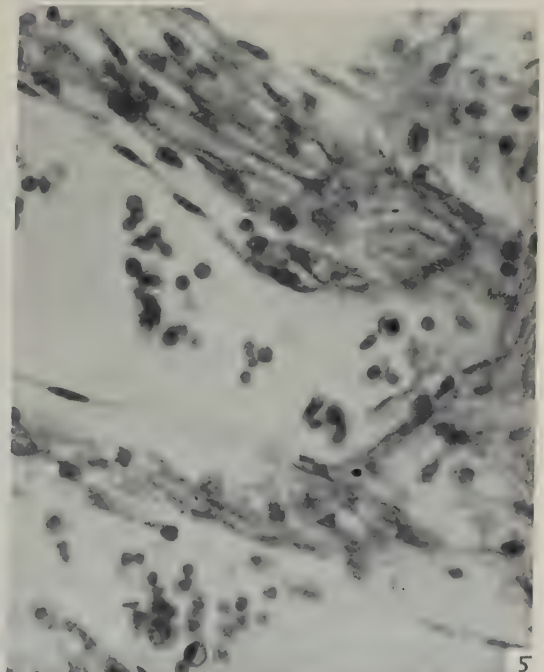
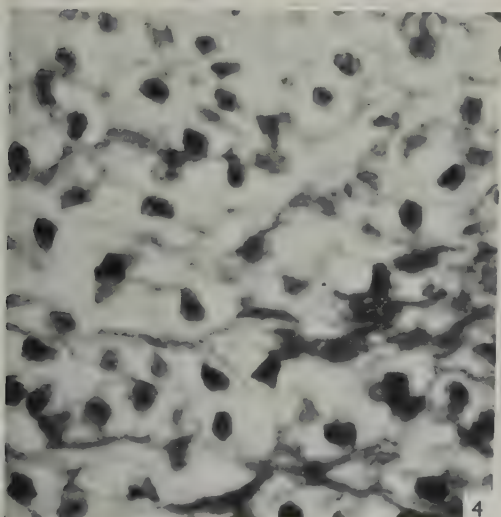
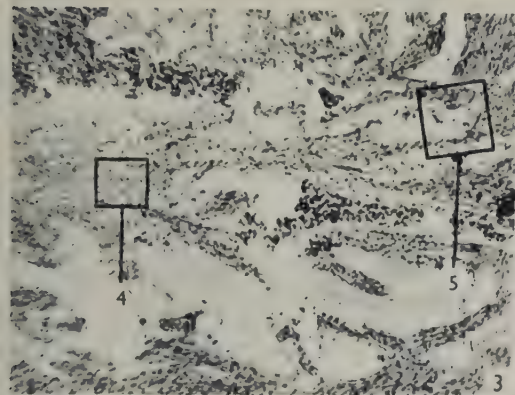
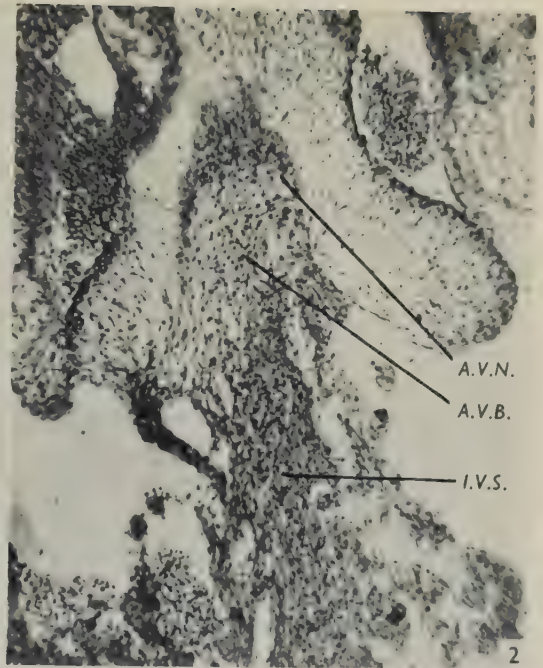
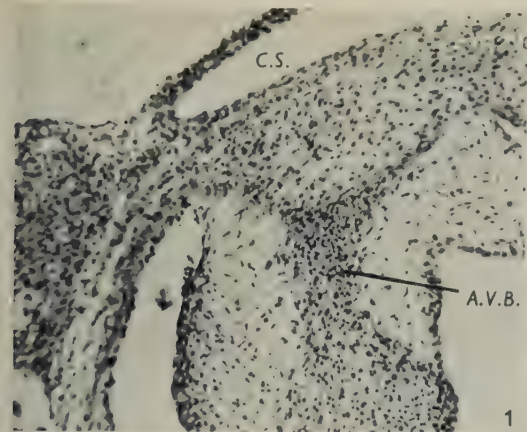
*A.V.N.*, atrioventricular node; *A.V.B.*, common trunk of atrioventricular bundle; *L.*, left branch of atrioventricular bundle; *I.V.S.*, interventricular septum; *N.*, nerve cells; *N.T.*, nerve trunk; *T.*, transition from modified to unmodified myocardium; *C.S.*, coronary sinus. All the figures are photomicrographs of sections of sheep's hearts stained by Hollande's chlorcarmine method.

#### PLATE 1

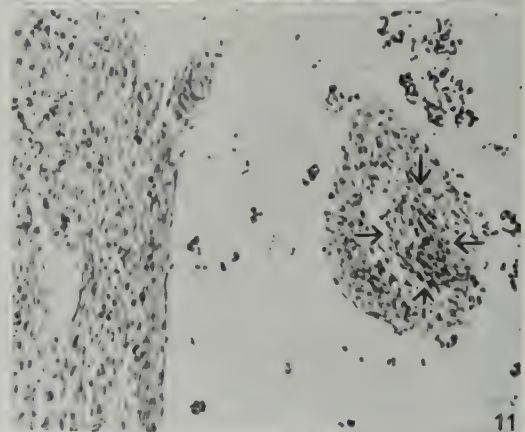
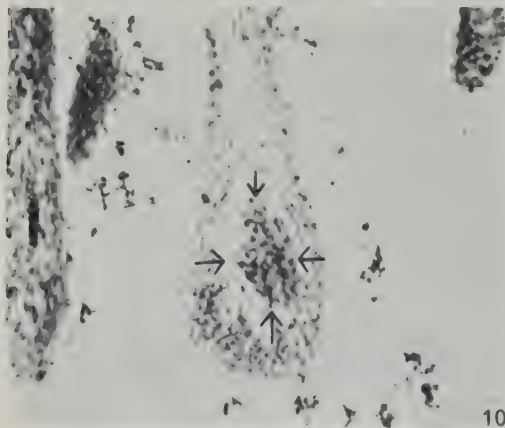
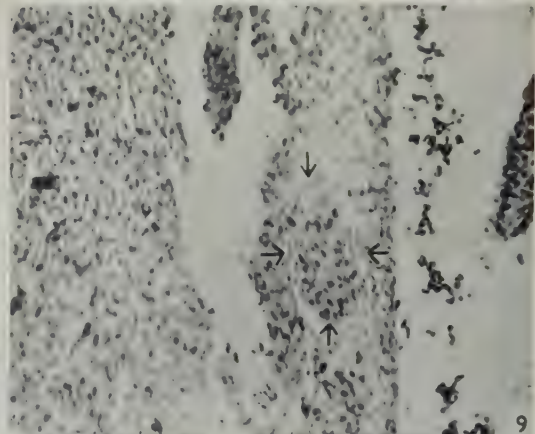
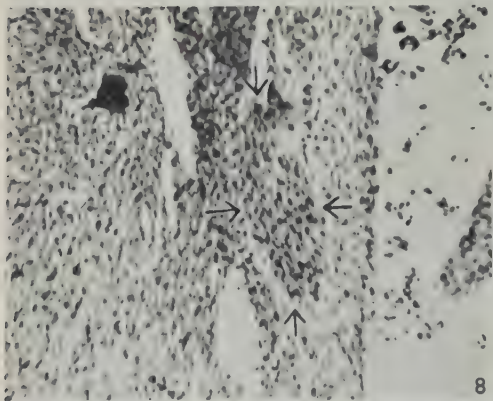
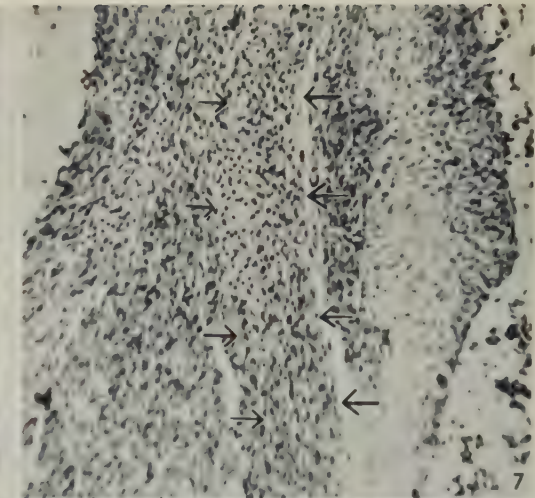
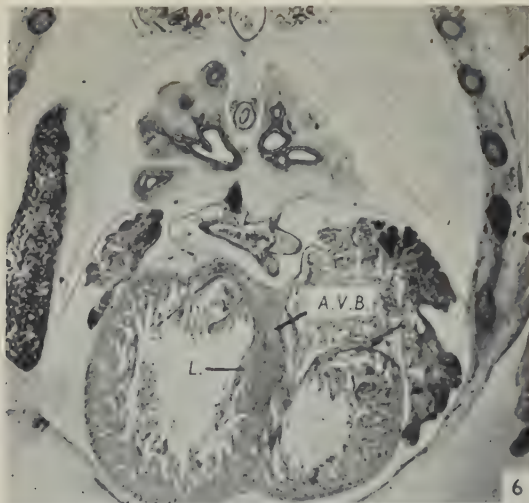
- Fig. 1. 10 mm.: the common trunk of the bundle can be seen running through the dorsal endocardial cushion. At the upper end, the bundle is continuous with the atrial muscle, in front of the coronary sinus, without the intervention of any specialized structure. The right atrioventricular function can be seen in the left of the photograph.  $\times 92$ .
- Fig. 2. 12 mm.: the atrioventricular node can be observed at the upper end of the common trunk of the bundle. The continuity between the paler cells of the bundle and the normal muscle of the interventricular septum can be seen.  $\times 92$ .
- Fig. 3. 18 mm.: this section is sagittal and passes tangentially to the left side of the interventricular septum. The broad, flat left branch of the bundle can be seen in the left of the picture, and numerous strands run to the right to be continuous with the mural myocardium.  $\times 40$ .
- Figs. 4 and 5. These photographs are taken from the upper and lower ends of one of the strands seen in fig. 3. The differentiated cells of the upper end have pale cytoplasm and spherical chromatic nuclei, and these fibres are continuous with the normal ventricular myocardium seen in fig. 5.  $\times 500$ .

#### PLATE 2

- Fig. 6. 20 mm.: section number 5.1.8 cut at  $10\mu$ ; section transverse to the thorax. The left ventricle is seen on the left and the right atrium and ventricle on the right. The slightly paler common trunk of the bundle can be seen in the upper part of the interventricular septum and the beginning of the left branch is seen close to the septum in the left ventricular cavity.  $\times 10$ .
- Fig. 7. The upper part of the interventricular septum from the low power view of fig. 6.  $\times 100$ . The fibres of the common trunk and proximal part of the right branch are modified in that they are pale, broad and their nuclei are densely stained. If the right branch is traced in this

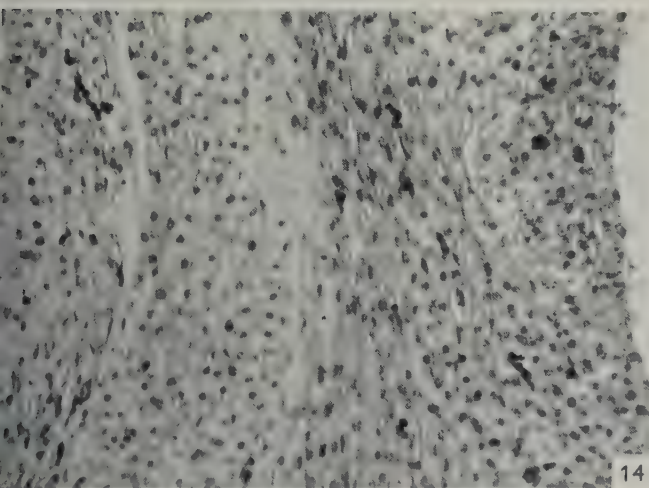
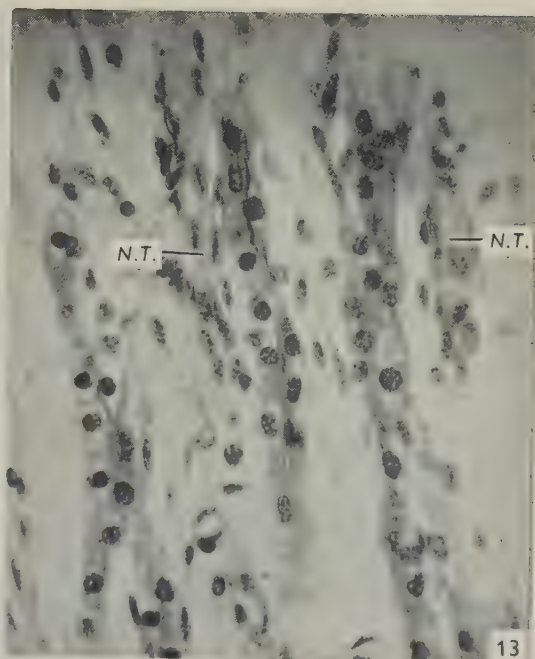


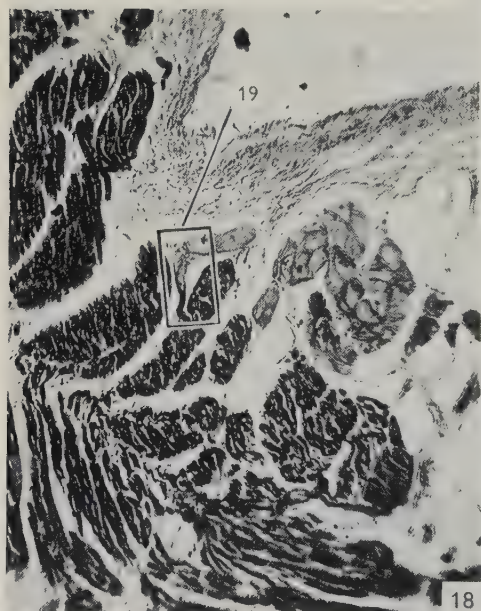
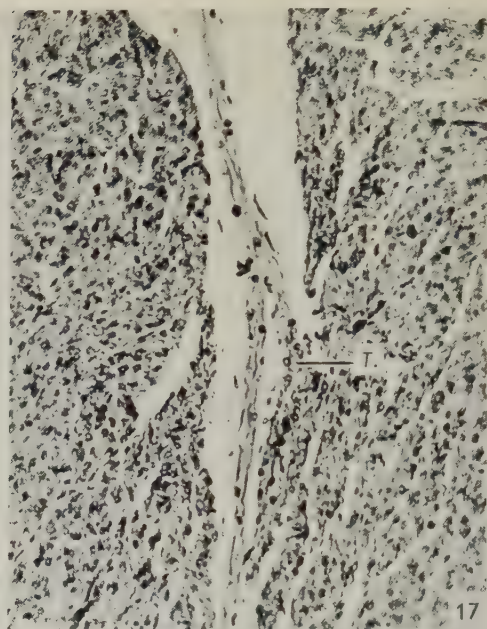
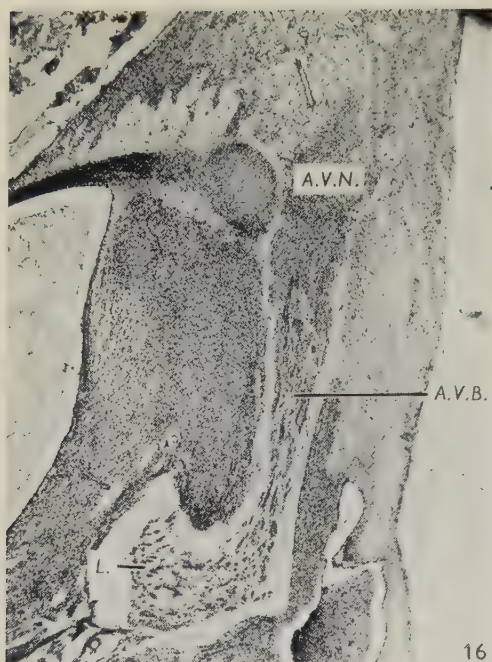




MUIR—DEVELOPMENT OF THE CONDUCTING TISSUE IN THE HEART OF THE SHEEP







and in the succeeding sections, it can be seen that the modified strands become continuous with an unmodified but discrete bundle. Although this bundle is cytologically indistinguishable from the myocardium of the interventricular septum, its discrete compact nature permits it to be followed into the moderator band.

Fig. 8. Section 5.1.4:  $\times 100$ .

Fig. 9. Section 5.1.2:  $\times 100$ .

Fig. 10. Section 4.3.6:  $\times 100$ .

Fig. 11. Section 4.2.5:  $\times 100$ .

PLATE 3

Fig. 12. 92 mm.: the septal cusps of the mitral and tricuspid valves can be seen on the left and right respectively. The short, broad common trunk of the bundle can be seen dividing at the lower end; compare with fig. 16; nerve cells can be seen in the interatrial septum above and to the left of the atrioventricular node.  $\times 35$ .

Fig. 13. 210 mm.: a high-power view of the cells of the common trunk from the section shown in fig. 16. There are three strands of Purkinje fibres and two nerve trunks shown in this field.  $\times 400$ .

Fig. 14. 42 mm.: the clearly differentiated common trunk can be seen passing between the normal muscle of the interventricular septum. A connective tissue sheath surrounds the bundle.  $\times 200$ .

Fig. 15. 72 mm.: a strand of young Purkinje fibres lies beneath the endocardium of the right ventricle near to the attachment of the moderator band. Transitions to the normal myocardium can be seen on the left of the picture.  $\times 160$ .

PLATE 4

Fig. 16. 210 mm.: the mitral valve cusp and the aortic vestibule are seen on the left, and the tricuspid valve on the right. The compact atrioventricular node can be seen to the right of the fibrous trigone, the continuity between the atrial muscle and the node can be seen. The attenuated common trunk of the bundle runs down from the node with the left branch separating off at the lower end. Compare with figs. 12 and 13.  $\times 25$ .

Fig. 17. Full term: a strand of Purkinje fibres is passing amongst the normal myocardium of the left ventricular wall. A transition to normal myocardium can be seen to the right of the strand of Purkinje fibres.  $\times 180$ .

Fig. 18. Adult: a subendocardial group of Purkinje fibres shows one transition to normal cardiac muscle.  $\times 100$ .

Fig. 19. Adult: a high-power view of the transition shown in fig. 18. Note the continuity of the myofibrils from the Purkinje fibre into the normal myocardium. A fibroblast nucleus is on the left of the Purkinje fibre and the connective tissue sheath of the Purkinje fibre continues around the normal muscle fibre.  $\times 1000$ .



## THE DEVELOPMENT OF TOOTH GERMS ON THE CHICK CHORIO-ALLANTOIS

BY SHIRLEY GLASSTONE

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Developing dental tissues of the cat, dog, guinea-pig, mouse, rabbit and man of embryonic and post-foetal stages have been grown experimentally in a number of ways. The first of these studies was undertaken as early as 1874 by Le Gros & Magitot, and since then a number of workers have entered this field and Table 1 summarizes the types of experiments which have been performed.

The results of these experiments, which include heterografts, autografts, homografts, xenografts and the use of the tissue-culture method, are strikingly uniform. Whole tooth germs continue to develop almost normally; the isolated enamel organ dedifferentiates or keratinizes; the dental pulp without odontoblasts does not develop further, whereas in the isolated pulp with odontoblasts, irregular dentine is formed (irregular plaques; serpiginous dentine, Huggins, McCarroll & Dahlberg, 1934). It has thus been concluded that the presence of the enamel organ is essential for the differentiation of the odontoblasts and for the maintenance of the normal shape of the tooth. It was claimed by Studitsky (1939) that the epithelia of the host chorio-allantoic membrane could act in the same way.

There is some variation in the extent to which isolated whole tooth germs develop. Although dentine has been formed in all instances, the production of enamel is more variable. Ameloblasts do not differentiate until the dentine has appeared and, in their absence, no enamel is formed. In tissue cultures, ameloblasts differentiate, but more than the earliest stages of the development of enamel are rarely observed, whereas appreciable layers of prismatic enamel have formed in grafted tooth germs (Huggins *et al.* 1934; Willis, 1935; Fleming, 1952*a*, 1953). An abnormal type of dentine was formed in some instances in tissue culture (Glasstone, 1936); again where grafts were left undisturbed for long periods, osteodentine was formed (Huggins *et al.* 1934; Fleming, 1952*a*), and in some grafted teeth only osteodentine formed (Santone, 1939; Villafane & Brero, 1942; Villafane & Gonzalez, 1942).

The main interest of most of the workers quoted in the table was to see if the tooth rudiments remained alive, and only a few of them gave secondary attention to the general morphological development. A more detailed study of this particular aspect of tooth development has been made by Glasstone (1938), who showed that the normal cusp pattern was developed in both rat and rabbit molars explanted before cusps had appeared. In a later paper (1952) she found that in halved tooth germs, regulation occurred, and each half formed a small but complete molar with a full set of cusps.

Grafting of dental tissues on to the chorio-allantoic membrane merits further attention than this technique has previously received. Other mammalian tissues

Table 1

Author	Nature of graft	Donor	Region for development of graft	Host
Le Gros & Magitot (1874)	(a) Whole teeth; (b) enamel organ; (c) portions of maxilla	Young dog	Subcutaneously	Guinea-pig (heterografts); dog (homografts)
Huggins <i>et al.</i> (1934)	(a) Enamel organ; (b) pulp with and without odontoblasts	Young dog	Subcutaneously	Young dog (autografts)
Willis (1935)	Minced-up jaws	Rat embryo	Brain	Rat (homografts)
Kostecka (1938)	(1) Whole teeth	Dog embryo	Subcutaneously	Adult dog (homografts)
Kostecka (1938)	(2) Whole teeth	Dog	Alveolar socket	Dog (autografts)
Kostecka (1938)	(3) Minced-up jaws	Mouse embryo	Brain	Mouse (homografts)
Sutro & Pomerantz (1939)	(a) Enamel organ; (b) pulp	Kitten	Marrow cavity of long bone	Kitten (autografts)
Santone (1939)	Whole teeth	Guinea-pig	Liver, kidney, ear	Guinea-pig (homografts and autografts)
Studitsky (1939)	Pulp without odontoblasts	Rat	Chorio-allantoic membrane	Chick (xenografts)
Lapchinsky & Malinowsky (1940 <i>a, b, c</i> , 1943)	(1) Whole teeth	Dog	Marrow cavity of long bone	Dog (autografts)
Hahn (1941)	(2) Whole teeth	Young dog	Alveolar socket	Dog (homografts)
Villafane & Gonzalez (1942); Villafane & Brero (1942)	Pulp	Young dog	Ovary	Dog (homografts)
Villafane & Gonzalez (1942)	Whole teeth	Rabbit	Subcutaneously	Rabbit (homografts)
Shapiro & Mclean (1945)	Whole teeth	Cat	Alveolar socket	Rabbit (homografts)
Fleming (1952 <i>b</i> )	(1) Whole teeth	Mouse embryo	Alveolar socket	Cat (homografts)
Fleming (1952 <i>a</i> )	(2) Whole teeth	Mouse embryo	Subcutaneously	Adult mouse, guinea-pig (homografts)
Fleming (1952 <i>b</i> )	(3) Whole teeth	Rabbit, mouse, guinea-pig and human embryos	Eye chamber	Rabbit, mouse, guinea-pig (homografts and heterografts)
Fleming (1952 <i>b</i> )	(3) Whole teeth	Mouse and rat embryos	Brain	Mouse and rat (homografts)
Glasstone (1936)	(1 <i>a</i> ) Whole teeth	Rat embryo	As tissue cultures	—
Glasstone (1936)	(1 <i>b</i> ) Pulp with and without odontoblasts	—	As tissue cultures	—
Glasstone (1938)	(2) Whole tooth-germs	Rat and rabbit embryos	As tissue cultures	—
Glasstone (1952)	(3) Halved tooth-germs	Rabbit embryos	As tissue cultures	—
Nuckolls (1941)	Whole teeth	Rat and mouse embryos	As tissue cultures	—
Loose (1943)	Whole teeth	Rat and mouse embryos	As tissue cultures	—
Szabo (1954)	(a) Whole teeth; (b) pulp; (c) enamel organ; (d) calcified tissues	Rat and mouse embryos and adults	As tissue cultures	—

grafted in this way have been shown to be vascularized by chick vessels (Nicholas & Rudnick, 1933). The present series of experiments were begun in order to study mammalian tooth development under these experimental conditions, with special reference to the formation of enamel, and by comparing the growth of whole tooth germs on the chorio-allantois with that previously obtained in tissue culture, to estimate more closely the importance of vascularization in the development of teeth.

#### MATERIALS AND METHODS

The first and second molars of foetal rats, mice and golden hamsters were used as grafts (Pl. 1, fig. 1; Pl. 2, fig. 8). Those of rats and mice were taken between 18 and 21 days of development, when cusps have appeared in the first but not the second molars. Odontoblasts are present on the cusps of the first molars taken at 21 days (Glasstone, 1938). In the golden hamster the equivalent stages of development are reached between 14 and 16 days. Thus the molar teeth were taken at roughly the same stage in all three species, though the degree of differentiation varies slightly both in different litters of the same age, and also in members of the same litter. Molars used for grafting were dissected out with sterile precautions and placed in Tyrode saline while the host eggs were being prepared for grafting. Either the first molar was dissected out by itself, or the first and second molars were taken together, joined by the intervening connective tissue. The developing molars on the opposite side of the jaw were used as controls, and were either left in position or were dissected out before fixation in Bouin's fluid or Acetic Zenker. The technique of chorio-allantoic grafting has been described by Murphy & Rous (1912).

Hens' eggs of 8 days' incubation were used as hosts. The eggs were candled and the site where two allantoic vessels met was marked. At this spot, a window, about 4 mm. square, was cut in the shell, by means of a fine carborundum disc rotated by a dental engine. This piece of shell was lifted off and the underlying membrane was flapped back. The tooth germs were transferred in a pipette, and were placed as near as possible to a blood vessel. The flap of shell membrane was returned, and over it the shell was replaced and waxed down. The eggs were then placed in the incubator with the grafts downwards and were left in this position for 24 hr., after which they were turned twice daily for 10 or 11 days. Grafts cannot be left on the chorio-allantois later than 19 days of incubation of the host, for after this time the membrane dries up as the beak of the embryo pierces the inner shell membrane, and the chick begins to breathe air. A circular cut was made in the shell 1-2 cm. round the original opening. This piece of shell, together with the underlying chorio-allantoic membrane, was placed in normal saline. A search was then made for the graft which, when not resorbed, was usually found near its original position. The graft was cut out, fixed in Bouin's fluid, dehydrated, and infiltrated with paraffin wax. Sections were cut at  $5\mu$  and stained in azan. One hundred and five grafts were made and thirty of these survived.

#### OBSERVATIONS

The development of the grafted molar teeth was normal in most respects (Pl. 1, figs. 2-5; Pl. 2, figs. 10, 11). A capillary circulation in the pulp was re-established, chick blood cells could be seen in the vessels, and the whole tooth became surrounded



by a dense plexus of dilated vessels (Pl. 1, figs. 3, 5). In the second molar, cusps in their normal pattern formed in the experimental period. Odontoblasts differentiated, and produced dentine normal in structure with tubules penetrated by the Tomes processes of the odontoblasts (Pl. 2, fig. 12). The maximum thickness of dentine in some grafts was about  $28\mu$ . The cells of the internal enamel epithelium differentiated into typical elongated ameloblasts with their nuclei adjacent to the stratum intermedium. These ameloblasts then began to produce enamel which in favourable instances formed, in the first molar, a layer up to  $25\mu$  (Pl. 1, figs. 2, 4 and 5), though in the second molar the layer of enamel was much thinner (Pl. 1, fig. 3). The normal prismatic structure of the enamel was evident, although in some instances it was distorted during fixation (Pl. 2, fig. 13). In azan sections the enamel stained a brilliant red in contrast to the dentine in which the collagen took up the aniline blue.

Of particular interest were the changes observed in the enamel organ whereby the stellate reticulum was invaded by host blood vessels and connective tissue. The degree to which this change occurred depended on the level of development of the tooth, and was proportional to the thickness of the layer of enamel. In the first molar where a thick layer of enamel formed, hardly any stellate reticulum remained (Pl. 1, figs. 2, 4 and 5; Pl. 2, fig. 10), but in the second molar which develops later than the first molar, a variable amount of stellate reticulum was present (Pl. 1, fig. 3; Pl. 2, fig. 11). The stratum intermedium was not affected by these changes. In grafts where the tooth remained healthy and there was no histological differentiation, the enamel organ was not invaded by the host blood vessels. This correspondence between development under normal and experimental conditions extends to the details of the process. In the early stages of vascular invasion of the enamel organ of these rodents there is considerable extravasation of erythrocytes into the meshes of the stellate reticulum (Pl. 1, fig. 6; Pl. 2, fig. 9), as has been noticed by Addison & Appleton (1922) and Kingery (1924) in the rat. This feature was also evident in the grafted tooth germs, particularly in the second molar (Pl. 1, figs. 3, 7) which at the end of the period of growth on the chorio-allantois had reached the early phases of invasion of the enamel organ. The first molar had by then progressed beyond this stage (Pl. 1, figs. 2, 4 and 5; Pl. 2, fig. 10).

#### DISCUSSION

In the present work the stage of development reached by the grafted molars is considerably in advance of that experienced in previous work with tissue cultures (Glasstone, 1936, 1938, 1952; Nuckolls, 1941; Losee, 1943), particularly with regard to the development of a relatively thick layer of prismatic enamel in the grafted molars. The difference in the general results of the tissue culture method and the chorio-allantoic grafts is presumably due to the presence of a circulation in the latter. Although some workers, who have grafted developing teeth into various sites in the body, have stated that the grafts became vascularized by the host capillaries, none of them have discussed the vascularization of the enamel organ, and in particular in those species in which it is known that this happens at an early stage in tooth development. We may thus consider to what extent the formation of normal enamel is conditional on vascularization. Green (1937), in his admirable paper on the teeth

of *Ornithorhyncus*, gives a comprehensive review of the literature up to that date on the vascularization of the enamel organ. Although there has been considerable controversy on the question, Green says 'later workers have almost unanimously agreed that the stellate reticulum does become vascularized at an earlier or later stage of tooth development in the mammals'. It seems likely, however, that there is no uniform order in which enamel and vessels in the stellate reticulum make their appearance in different mammals (Jump, 1938).\*

In the normal development of molars in the rat, mouse and golden hamster, erythrocytes and blood vessels penetrate the enamel organ approximately 2-3 days before enamel forms. The vessels reach the stratum intermedium about 3 days after the dentine first appears. It has been stated that in the development of teeth in man (Kingery, 1924; Jump, 1938), and in the pig (Kingery, 1924), the blood vessels do not invade the enamel organ until a thin layer of enamel is present.

The extent to which capillaries penetrate into the enamel organ also varies in different species. Green (1937) concluded that in *Ornithorhyncus* 'enamel formation is dependent on the close relationship of the capillaries with the *stratum intermedium*...', whereas, to judge from Kingery's photographs of the human enamel organ, there is a relatively wide zone of stellate reticulum which remains non-vascular. There is more or less a general agreement that in the marsupials and Rodents, the formation of enamel is dependent on the presence of erythrocytes and blood vessels in the enamel organ. Apart from these species no detailed study of this relationship has been made. From time to time there has been observations on isolated stages of tooth development, yet it does seem that in some species the formation of enamel is not immediately dependent on the close proximity of the blood vessels. The fact that the development of tooth germs on the chick chorio-allantoic membrane proceeded further than in tissue culture is probably due to a better nutrient environment supplied by the vascular membrane.

From the facts discussed above, it can be concluded that the vascularization of a developing tooth is clearly controlled by internal factors. Erythrocytes or blood vessels do not invade the enamel organ until the stages of development appropriate to the species is reached. This conclusion is supported by the similarity in the degree of vascularization of the enamel organ in the normal development of the teeth of rat, mouse, and golden hamster, and of their development on the chorio-allantoic membrane. In the normal development of these species erythrocytes and blood vessels are present in the enamel organ a little before the enamel is formed. This order of development occurs in the grafts on the chorio-allantoic membrane, the erythrocytes and blood vessels from the membrane invading the enamel organ just prior to enamel formation. It has been previously shown, that the development of cusps in molar teeth is a self-differentiating process (Glasstone, 1938), and the above experiments are considered to reveal yet another aspect of self differentiation in tooth development. The chorio-allantoic vessels which invade the enamel organ, and the pulp, of these mammalian grafts, probably consist of avian endothelium,

\* Indeed the presence of erythrocytes or blood vessels in the enamel organ of certain mammals is still not easy to establish without specific staining methods. In my work on the rabbit (Glasstone, 1938) I was not able to convince myself that erythrocytes were present in this situation. Further observations are being made on the development of the enamel organ in this and other mammals.

and yet their effect on the grafts is the same as that of normal mammalian capillaries.

#### SUMMARY

1. The first and second molars of foetal rats, mice and golden hamsters have been grown as grafts on the chorio-allantoic membrane of the chick. These organs continued their development under these circumstances.

2. The grafts were made at a time when cusps had appeared in the first molar. In the second molar they developed during the period of grafting. In both teeth odontoblasts and ameloblasts differentiated and dentine and enamel of normal structure were formed.

3. Erythrocytes and blood vessels from the chorio-allantoic membrane invaded the pulp, and the enamel organ as far as the stratum intermedium, and behaved in each situation as in normal development.

4. The stellate reticulum was vascularized before enamel was formed. This process is probably controlled by factors intrinsic to the developing tooth germ.

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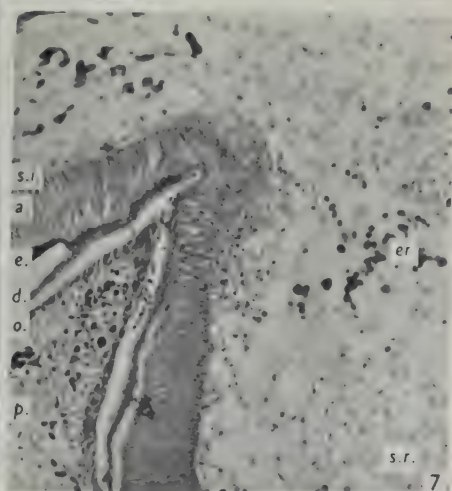
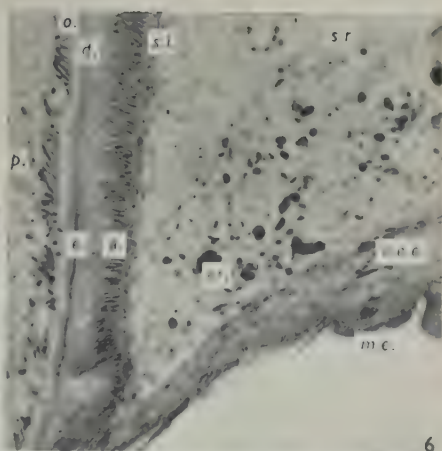
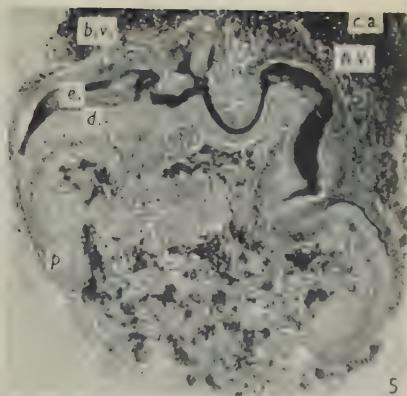
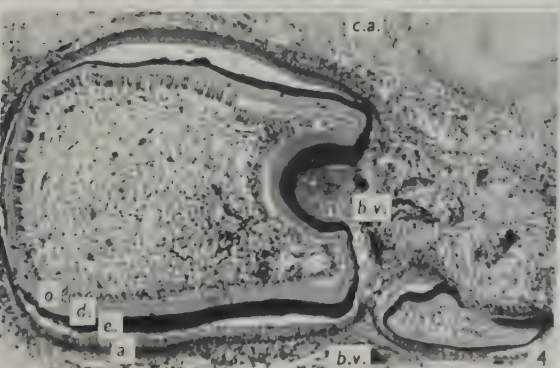
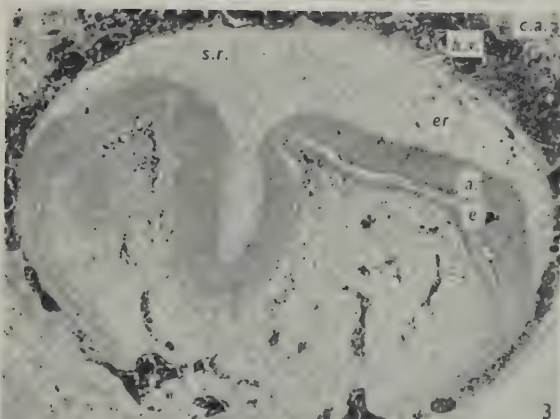
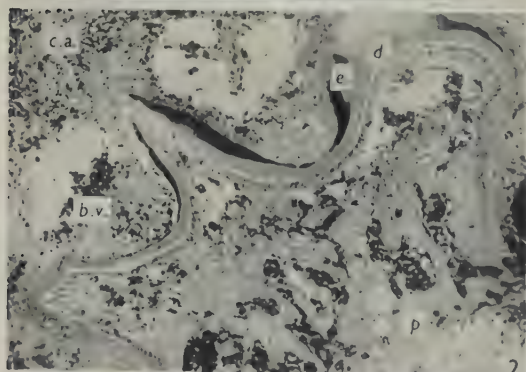
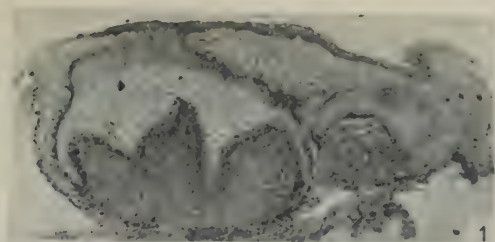
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#### EXPLANATION OF PLATES

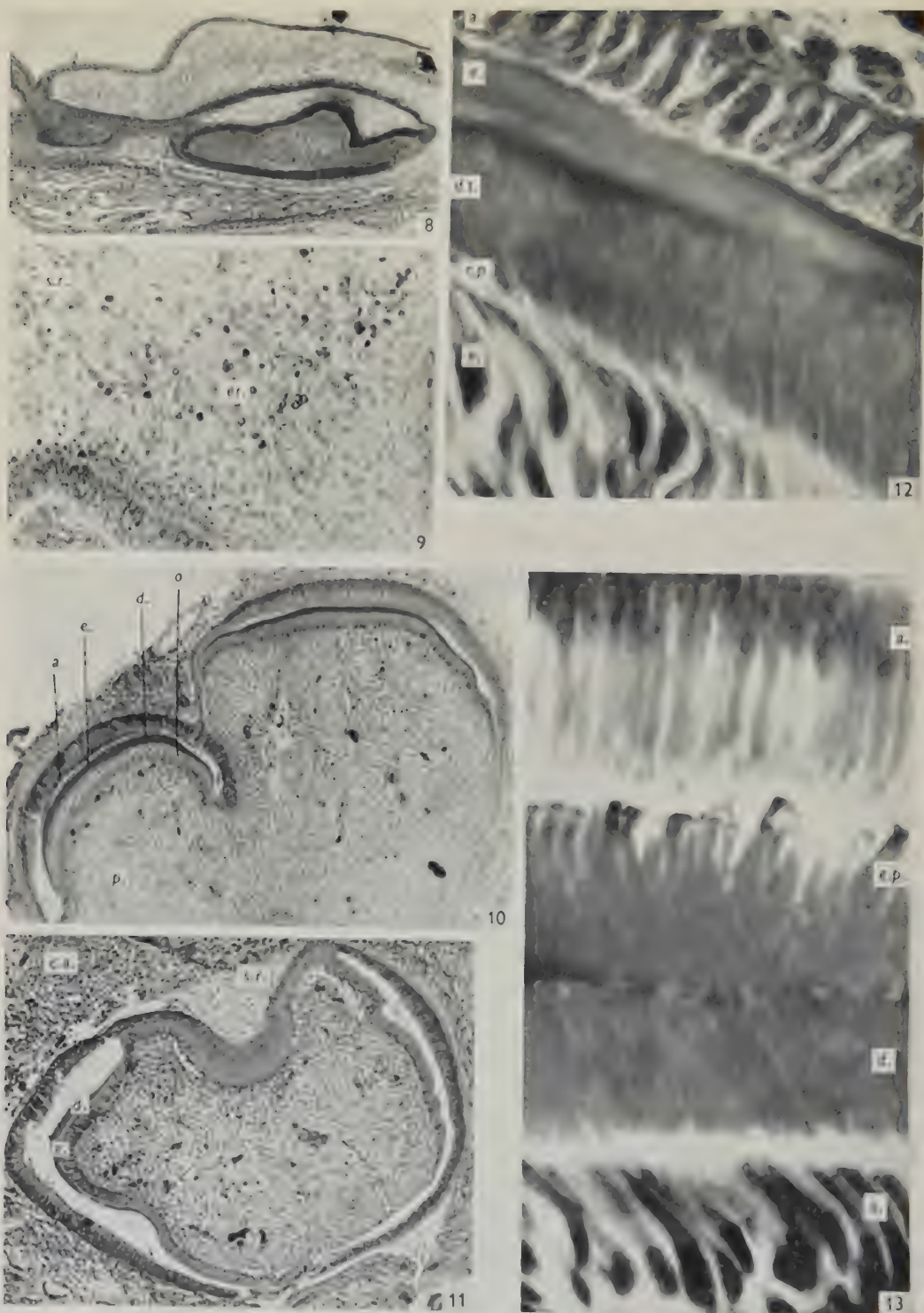
Sections through embryonic molar teeth of hamsters (Pl. 1), and rats (Pl. 2) stained by the Azan method. Figs. 1, 6, 8 and 9 are of normal stages; the others are of teeth grown on the chorio-allantoic membrane of the chick. The stages denoted are *post coitum*.

#### PLATE 1. HAMSTER MOLARS

- Fig. 1 Longitudinal section of the first and second molars at 15 days. The cusps are present in the first molar, but not in the second.  $\times 40$ .
- Figs. 2, 3. Longitudinal sections through 15-day first and second molars, grafted for a further 10 days. Both teeth were adjacent in the foetus and were grafted together. In fig. 2 the enamel organ has disappeared and has been replaced by host connective tissue. Dentine has formed and over it there is a layer of enamel. In this photograph the enamel is the darker layer. The host blood vessels have invaded the dental pulp.  $\times 72$ .
- Fig. 3. Cusps, odontoblasts and thin layers of dentine and enamel have formed. Capillaries and erythrocytes can be seen in the enamel organ over the right-hand cusp. The host blood vessels are massed round the outside of the tooth germ.  $\times 72$ .
- Fig. 4. Transverse section of the first molar at 15 days, grafted for a further 10 days. There are thick layers of dentine and enamel; the latter is the darker layer in the photograph.  $\times 72$ .
- Fig. 5. Longitudinal section of a 15-day molar grafted for 10 days. Notice continuous layers of dentine and enamel. The latter is the thicker and appears darker.  $\times 72$ .
- Fig. 6. Section of a control molar tooth of a hamster 1 day after birth to show the early stages of invasion of the stellate reticulum by capillaries and erythrocytes. The enamel is just beginning to form.  $\times 176$ .







GLASSTONE—THE DEVELOPMENT OF TOOTH GERMS ON THE CHICK CHORIO-ALLANTOIS



Fig. 7. Section of a 15-day second molar grafted for 10 days, to show the early invasion of the stellate reticulum by erythrocytes, as in the previous figure. There is a thin layer of darkly stained enamel.  $\times 176$ .

PLATE 2. RAT MOLARS

Fig. 8. Longitudinal section of the first and second molars at 19 days. The cusps are present in the first, but not the second molar.  $\times 40$ .

Fig. 9. Section of first molar of a 21-day rat to show the invasion of the stellate reticulum by blood vessels and erythrocytes.  $\times 176$ .

Figs. 10, 11. Longitudinal sections through 19-day first and second molars, grafted for a further 10 days. Both teeth were adjacent in the foetus and were grafted together. In Fig. 10 the enamel organ has disappeared and been replaced by host connective tissue. Dentine has formed and a thin layer of enamel over it. The host blood vessels have invaded the dental pulp. In fig. 11 cusps, odontoblasts, a thin layer of dentine are present and the enamel is just beginning to appear. The enamel organ has not yet been fully replaced by host connective tissue.

Fig. 12. Part of a section of the same tooth as in fig. 10 at a higher magnification, to show ameloblasts above, odontoblasts below and enamel and dentine between them. Notice the dentine tubules with the Tomes processes from the odontoblasts.  $\times 1024$ .

Fig. 13. Part of a section of the same tooth as fig. 10. The ameloblasts are above and the odontoblasts below with the enamel and dentine between. Notice the prismatic structure of the enamel.  $\times 1024$ .

ABBREVIATIONS

<i>a.</i>	ameloblasts	<i>er.</i>	erythrocytes
<i>b.v.</i>	blood vessels	<i>m.e.</i>	mouth epithelium
<i>c.a.</i>	chorio-allantoic membrane	<i>o.</i>	odontoblasts
<i>d.</i>	dentine	<i>p.</i>	pulp
<i>d.t.</i>	dentinal tubules	<i>s.i.</i>	stratum intermedium
<i>e.</i>	enamel	<i>s.r.</i>	stellate reticulum
<i>e.e.e.</i>	external enamel epithelium	<i>t.p.</i>	Tomes process
<i>e.p.</i>	enamel prisms		

# A COMPARATIVE STUDY OF THE AZYGOS VENOUS SYSTEM IN MAN, MONKEY, DOG, CAT, RAT AND RABBIT

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It has been generally considered that the azygos vein acts as a by-pass between the inferior and superior caval systems, and most research has centred on the connexions of its caudal end. In view of the correlation between changes in the pressures of the azygos vein and the cerebrospinal fluid, it was decided to investigate the functional value of the azygos venous system, and the importance of its connexions with the internal vertebral venous system.

## HISTORICAL INTRODUCTION

The azygos vein has excited interest since the earliest days of anatomical study, and is mentioned in the third century by Galen (ed. 1822). Vesalius (1555) wrote of it at some length, and alluded to its connexion with the internal vertebral veins and inferior vena cava. Eustachius (1722) shows, without comment, the hemiazygos arising from the left renal vein. Winslow (1776) gives a fairly accurate description of the vein, together with the branches draining the spinal canal into the intercostal veins.

The definitive anatomy of the spinal (internal vertebral) veins and their connexions was established by the Paris school in the first half of the nineteenth century, and undoubtedly the most important of these writers was Breschet (1829). He pointed out that a large vein emerges from each thoracic intervertebral foramen and ascends over the body of the vertebra to join the azygos or hemiazygos vein, having first joined forces with the posterior intercostal vein; and that this 'vein of the intervertebral foramen' is larger than the intercostal vein which overlies it. The description of the azygos and internal vertebral venous systems in the textbook of Poirier & Charpy (1902) is based mainly on the work of Breschet (1829) and of Walther (1885).

These authors, and Breschet independently of them, state that there are no valves in the internal vertebral veins or in the azygos or hemiazygos, except that there is sometimes an incompetent valve in the arch of the azygos. It should be mentioned, however, that there is a valve in the posterior intercostal vein just before it joins the vein of the intervertebral foramen.

Research has centred mainly upon the tenuous connexions of the caudal end of the azygos system, starting with Lejars's description of the 'canal réno-azygo-lombaire' (1888) said to be present in 80 % of cases. Seib (1934) dissected 100 white and 100 negro cadavers and classified the inferior caval and renal origins of the azygos and hemiazygos veins. Analysis of his tables shows that in 83 % of cases the azygos is connected with the inferior vena cava or the left renal vein.

The azygos system has also been studied by authors whose main interest was in the collateral circulation following obstruction of the inferior vena cava. Among the more important of these are Sappey & Dumontpallier (1861), Pleasants (1911) and Batson (1940). Recently, Robinson (1949) has studied the collateral circulation in stillborn foetuses after ligation of the inferior vena cava, and has indicated the connexions between the azygos and internal vertebral systems. Coman & de Long (1951) have made vinylite corrosion preparations in the rat showing the normal anastomoses between the azygos and internal vertebral systems. Herlihy (1948) has studied the internal vertebral venous plexus of the cat, demonstrating the topography and mentioning its connexions with the azygos venous system, to which he also refers in an earlier publication (1947).

Much of the literature on the internal vertebral venous system has been reviewed by Harris (1941).

#### MATERIAL AND METHODS

The material consisted of four full-term stillborn (but otherwise normal) human foetuses, three macaque (*Macaca mulatta*) monkeys, three dogs, two cats, twenty albino rats, and six rabbits. All the material except the human was adult. In all cases the azygos vein was injected retrogradely with either 20% bismuth oxychloride ('Chlorbismol') or a dispersion of barium sulphate ('Micropaque'), preparatory to radiography and dissection. Four of the rats underwent operative ligation of the vena azygos at its junction with the superior vena cava.

In the operative technique, positive pressure anaesthesia was provided by a modification of the method of Porter & Small (1947), details of which will be published elsewhere. The rat azygos is left-sided (see Beddard, 1907), so a lateral thoracotomy was performed in the third left intercostal space and the third rib resected. A ligature was passed under the azygos vein at its point of entry into the superior vena cava, immediately medial to the arch of the aorta, and the vein tied off. The chest was then closed in layers without drainage. These rats were killed and injected at intervals between 10 days and 1 month after operation. Once the anaesthetic and operative technique had been perfected, the mortality was nil.

In the earlier cases of the series, the animals were heparinized before being killed, in order to facilitate the flow of the injection material; but experience showed that, provided the injection was made immediately after death, this made no difference.

In all cases of retrograde injection of the azygos vein, the following ligations were made before injection:

- (i) Inferior vena cava, cephalic to the renal veins, but below the liver.
- (ii) Inferior vena cava, between the diaphragm and heart.
- (iii) Both renal pedicles at the hilum.
- (iv) Both lung roots.
- (v) Inferior vena cava at its point of formation from the common iliac veins.

The liver, gut and both lungs were removed before injection, and the abdominal vena cava between the two lower ligatures was removed after injection.

In a few specimens (e.g. cat, Pl. 3, figs. 7, 8), towards the end of the injection, the injection mass burst through into the surrounding connective tissue between the layers of the mediastinal pleura, but this did not affect the radiographic pattern.



## RESULTS

In all the species studied, the azygos and hemiazygos veins were well filled with injection mass. The degree of filling of the posterior intercostal veins was variable, depending upon the force of the injection and the competence of their valves. In the cases in which they did fill, it could be observed by direct vision that they were the last vessels to be filled, and injection was immediately stopped if and when the veins filled.

In the human foetuses (Pl. 2, figs. 3, 4) the intercostal veins were never in any case filled. This is testimonial to the statement of Poirier & Charpy (1902) that venous valves are more competent in infancy.

Also in each species can be seen, at least in the thoracic region, in dorso-ventral views, the 'vein of the intervertebral foramen' joining the posterior intercostal vein just prior to its termination. In most cases it can be seen that this vessel is larger than the posterior intercostal vein prior to this union.

Three other features of importance were noted in all species. First, in no case, save in the monkey, was any segment of the inferior vena cava filled by the injection mass. Even in the monkey, this was probably due to the fact that the left renal vein had been ligated lateral to its suprarenal tributary.

Secondly, in all species (though not all specimens examined), the left suprarenal gland was outlined not through the suprarenal-renal vein, but by a vessel which issues from the first lumbar intervertebral foramen and joins the plexus of vessels issuing from the gland.

Lastly, in all species some or all of the torn ends of the lumbar veins were filled.

In the Primates and Carnivora examined the internal vertebral venous plexus was constantly filled. In the four species studied, the main vessels seen are two lateral longitudinal trunks, whose position in relation to the vertebral bodies can be estimated by comparison of dorso-ventral and lateral radiographs. Dissection showed that they lay in the vertebral canal, outside the dura mater in the epidural fat. They received a large number of tributaries which emerge from the dura in company with the nerve roots. Certain differences in these veins between the Primates and Carnivora can be observed. Thus, in the Primates (Pls. 1, 2, figs. 1-4) there are a large number of cross-connexions between these two lateral longitudinal trunks, and the communications with the intradural vessels appear to be much richer than in the case of the Carnivora. In the cat (Pl. 3, figs. 7, 8) there are no cross-connexions between the lateral longitudinal trunks; this confirms the observations of Herlihy (1948). In the dog, the cross-connexions are very sparse (Pl. 2, figs. 5, 6). In the Primates, the internal vertebral venous plexus connects at its lower end with the anterior sacral plexus (Pl. 1, fig. 1; Pl. 2, fig. 3), which lies on the anterior aspect of the bodies of the sacral vertebrae; this plexus is not apparent in the Carnivora. Inspection of the lateral radiographs (Pl. 1, fig. 2; Pl. 2, fig. 5; Pl. 3, fig. 8) shows the lateral longitudinal trunks to be greater in diameter than the vena azygos itself. It should also be noted here that man is the only species examined which appears to possess ascending lumbar veins.

In the normal rodent (Pl. 4, figs. 9, 10) the lower end of the azygos venous system terminates at the level of the left suprarenal vein; and although the connexions of

the azygos and hemiazygos veins with the veins of the intervertebral foramina in the thoracic region are apparent, the internal vertebral venous plexus is not filled.

However, after operative ligation of the azygos vein, flow in this system is reversed, and hypertrophy of the main trunks and all its tributaries occurs. Although this operation was successfully carried out in the rat, the rabbit seems unable to stand the procedures involved, and no rabbits survived operation. Injection of the operated rat (Pl. 4, figs. 11, 12) shows the presence of two lateral longitudinal trunks (less well defined than in Primates and Carnivora), with numerous tributaries, and also a dorsal longitudinal trunk, very evident in cleared specimens, which runs along the tips of the vertebral spines. Here again the lateral longitudinal trunks are larger (when filled under pressure) than the vena azygos major.

#### DISCUSSION

It would appear that the azygos venous system is the main intermediary between the superior caval system and the internal vertebral venous system. It is a fact of no little importance that it is the superior caval system which is mainly concerned. Many previous investigators (e.g. Seib, 1934) have been concerned with the connexions between the lower end of the azygos and hemiazygos veins with the inferior caval system. It is shown anatomically that these connexions are tenuous and of no functional importance. The most important piece of evidence in this connexion is the effect of ligation of the vein in the rat. There is no enlargement of any azygo-inferior-caval anastomoses; nor is there any effect upon the posterior intercostal vessels (Pl. 4, figs. 11, 12), which shows that in the thoracic cage of the rat internal vertebral connexions are of paramount importance, a point which was not stressed by Halpern (1953). Breschet (1829) was the first to claim that flow in the azygos and internal vertebral venous plexuses may be in either direction, and further attention has been drawn to this by Franklin (1937).

The recent work of Collins, Weinstein, Norton & Webster (1952) has demonstrated the collateral circulation following ligation of the inferior vena cava, and this has been studied by Robinson (1949). Previous to these workers, Sappey & Dumontpallier (1861) and Pleasants (1911) had studied the collateral circulation in such cases. They all stress the enlargement of the internal vertebral venous plexus and of the azygos major. In all such cases it would appear that inferior caval blood is first transferred to the internal vertebral venous plexus and thence to the azygos system.

Batson (1940) has stressed the importance of the internal vertebral venous plexuses in metastatic phenomena. It seems that by means of the connexions between the azygos and internal vertebral venous systems, an explanation may be found for the frequency of the occurrence of metastases from bronchial carcinoma to the adrenal gland; it is intended further to investigate this particular problem.

Lastly, the role of this venous plexus in the control of cerebro-spinal fluid pressure should be noted. This has already been the subject of some attention by Herlihy (1947) and Bowsher (1953). The filling of the lateral longitudinal sinuses and their tributaries causes an increase in cerebro-spinal fluid pressure; this filling can in its turn be caused by compression of the intrathoracic azygos veins, which squeezes blood into the internal vertebral venous plexus; and this appears to be the

mechanism of the respiratory variation in cerebro-spinal fluid pressure. Similarly, a rise in pressure is caused by inferior caval obstruction and this is compensated by adjustments in the production and absorption of cerebro-spinal fluid.

It is also important to note that the venous drainage of the adrenal is not solely into the renal vein on the left side. Attempts to measure the blood flow through, or hormonal output from, the left adrenal gland by cannulation of the left adrenal vein (e.g. Vogt, 1944) are probably not entirely accurate.

#### SUMMARY

1. The literature on the anatomy of the azygos vein and its connexions is reviewed.

2. The azygos venous system and its connexions, particularly with the internal vertebral venous plexus, is described in man, monkey, cat, dog, rat and rabbit.

3. It is particularly stressed that the azygos venous system is functionally a part of the internal venous vertebral system, draining this latter into the superior caval system. Various consequences of this function are discussed.

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## EXPLANATION OF PLATES

## PLATE 1

The normal pattern of the azygos and internal vertebral venous systems in the monkey (*Macaca mulatta*). After ligation of the inferior vena cava cephalic to the renal veins, but below the liver, the inferior vena cava between the diaphragm and heart, both renal pedicles at the hilum, both lung roots, and the inferior vena cava at its point of formation from the common iliac veins, and removal of the liver, the gut below the diaphragm and both lungs, the azygos vein was injected retrogradely with barium sulphate suspension ('Micropaque'), and the abdominal vena cava between the two lower ligatures was then removed prior to radiography.

Fig. 1. Dorso-ventral view. *A*, vein of intervertebral foramen; *B*, lateral longitudinal internal vertebral vein; *C*, suprarenal vein; *D*, anterior sacral venous plexus.

Fig. 2. Lateral view. *A*, azygos vein; *B*, lateral longitudinal internal vertebral vein; *C*, suprarenal vein, showing connexion to internal vertebral system; *D*, lumbar vein.

## PLATE 2

The normal pattern of the azygos and internal vertebral venous systems in the fresh stillborn full-term human foetus and in the dog, as shown by radiography after the ligations described in the legend to Plate 1.

Fig. 3. Human foetus. Dorso-ventral view. *A*, ascending lumbar vein; *B*, lateral longitudinal internal vertebral vein; *C*, anterior sacral plexus.

Fig. 4. Human foetus. Lateral view. *A*, azygos vein; *B*, lateral longitudinal internal vertebral vein.

Fig. 5. Dog. Lateral view. *A*, azygos vein; *B*, lateral longitudinal internal vertebral vein; *C*, suprarenal-vertebral vein; *D*, lumbar vein.

Fig. 6. Dog. Dorso-ventral view. *A*, lateral longitudinal internal vertebral vein; *B*, azygos vein; *C*, suprarenal-vertebral vein.

## PLATE 3

The normal pattern of the azygos and internal vertebral venous systems in the cat. In the upper part of the figures it can be seen that the injection mass has burst through the azygos vein into the surrounding connective tissue (mediastinal pleura), but the lower part of the vein is intact. Radiography after the ligations described in the legend to Pl. 1.

Fig. 7. Dorso-ventral view. *A*, azygos vein (which has burst into surrounding connective tissue); *B*, lateral longitudinal internal vertebral vein; *C*, suprarenal-vertebral vein.

Fig. 8. Lateral view. *A*, azygos vein, which has burst into surrounding connective tissue; *A'*, lower (unburst) portion of azygos vein; *B*, lateral longitudinal internal vertebral vein; *C*, suprarenal-vertebral vein; *D*, lumbar vein.

## PLATE 4

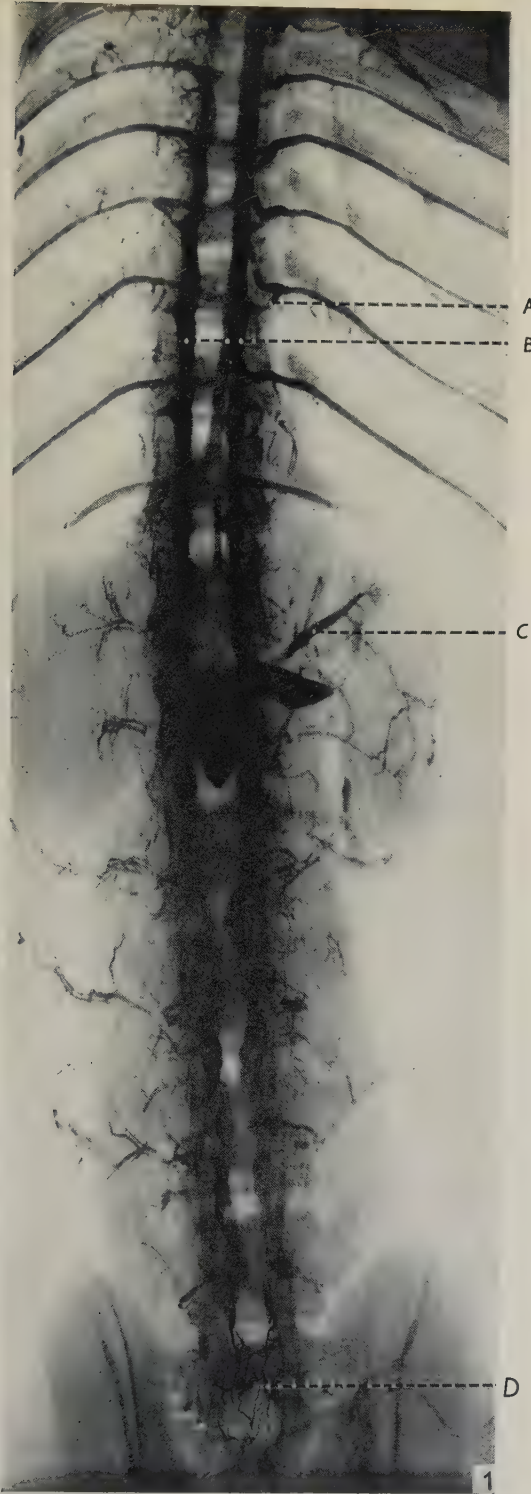
The normal pattern of the azygos and internal vertebral venous systems in the rabbit and rat (figs. 9, 10). The pattern of the azygos and internal vertebral venous systems in the rat 1 month after operative ligation of the azygos vein at its entry to the superior vena cava (Figs. 11, 12). Radiography after the ligations described in the legend to Pl. 1.

Fig. 9. Dorso-ventral view. *A*, azygos vein; *B*, vein of intervertebral foramen; *C*, lateral longitudinal internal vertebral vein; *D*, suprarenal-vertebral vein.

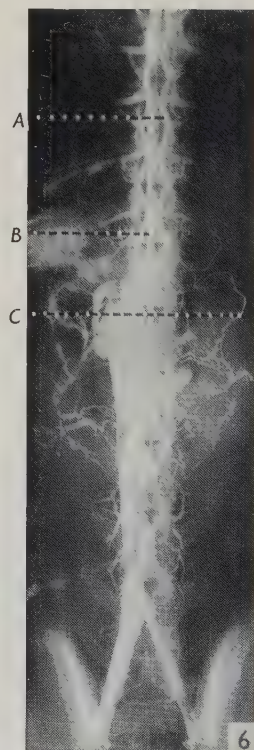
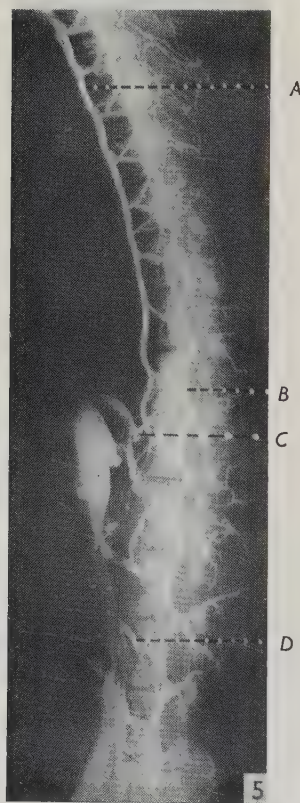
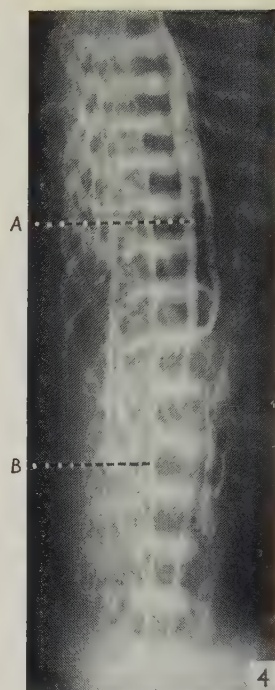
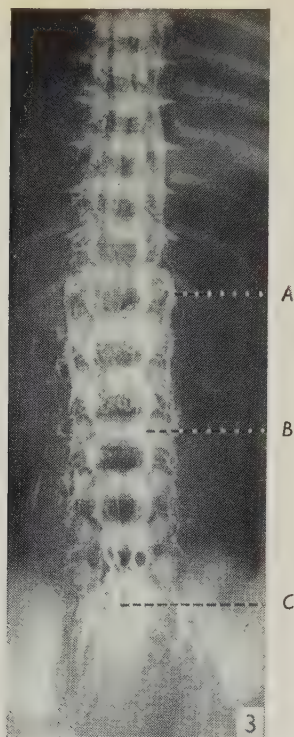
Fig. 10. Dorso-ventral view. *A*, vein of intervertebral foramen; *B*, azygos vein; *C*, lateral longitudinal internal vertebral vein; *D*, suprarenal vein; *E*, suprarenal-vertebral vein.

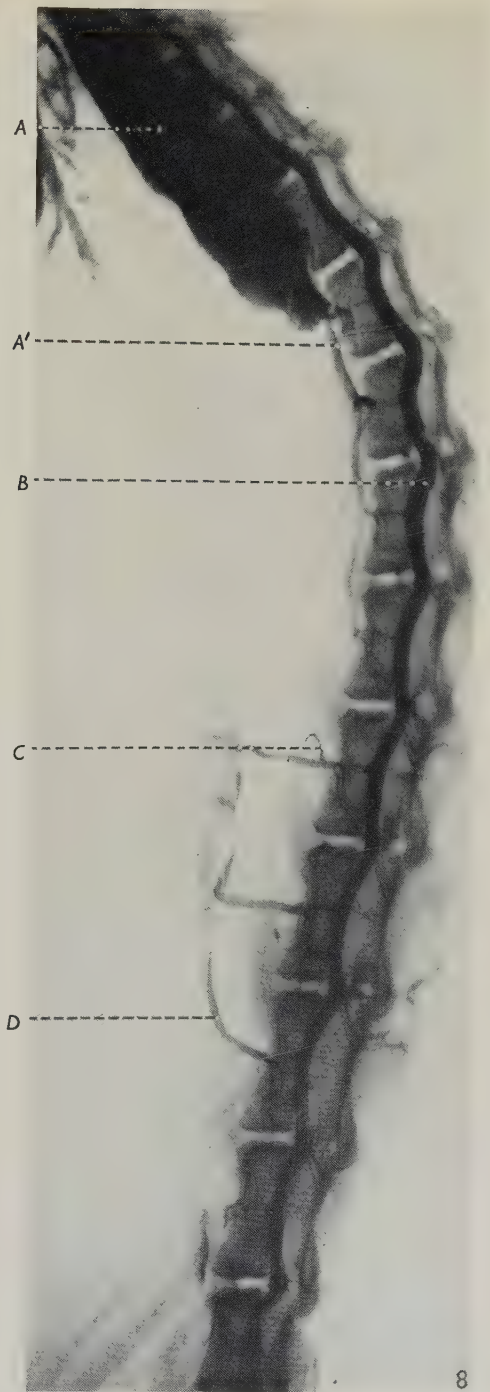
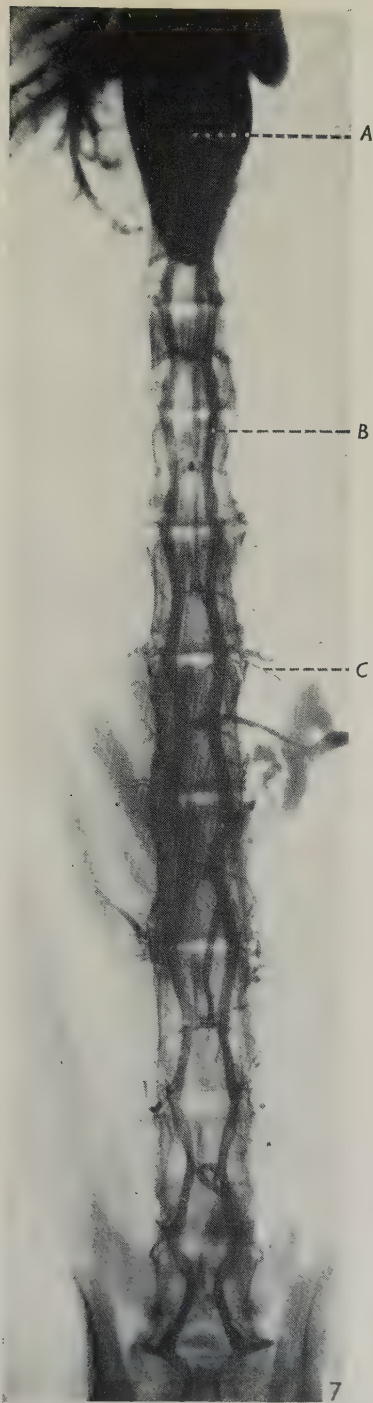
Fig. 11. Dorso-ventral view. *A*, vein of intervertebral foramen; *B*, lateral longitudinal internal vertebral vein; *C*, connecting vessel between the two lateral trunks.

Fig. 12. Dorso-ventral view. *A*, lateral longitudinal internal vertebral vein; *B*, suprarenal-vertebral vein; *C*, lumbar vein.

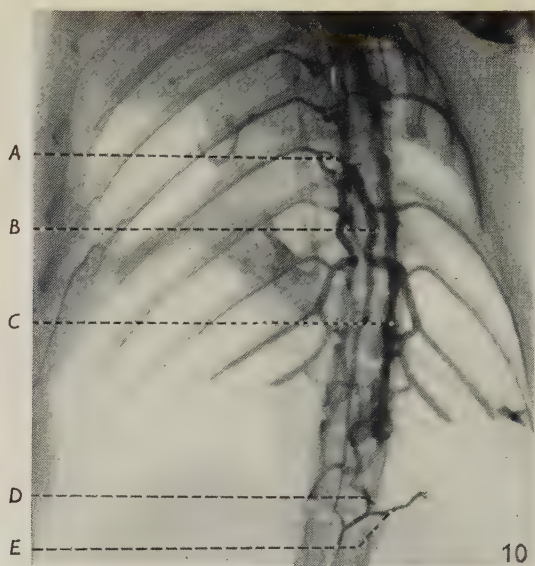
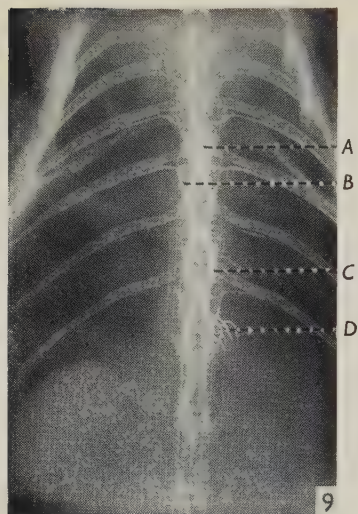














## A NOTE ON THE RADIOLOGICAL DEMONSTRATION OF THE PERIRENAL SPACE

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The perirenal space that is formed by the renal fascia is generally believed to be open below. The anterior layer of fascia (the fascia of Toldt) is also usually described as passing in front of the aorta and inferior vena cava to meet the corresponding fascia of the opposite side. This description has recently been questioned by Mitchell (1939, 1950), who writes, mainly on the basis of X-ray observations on cadavers, that the anterior layer of renal fascia fuses with the posterior (the fascia of Zuckerkandl) below the kidney, and medially with the areolar tissue around the great vessels. He also states that the weakest area of fusion is below, around the ureters. This picture of the disposition of the fascia would conform to the usual clinical observation that a perinephric abscess on one side of the body does not spread to the other. On the other hand, it does not fit certain radiological observations about the flow of gas introduced into the renal fascia in order to help delineate the kidneys and adrenals by X-rays.

The first of these techniques was introduced by Carelli (1921), who injected gas directly into the perirenal space. It is stated that gas so introduced can flow from one kidney capsule to the next (Mencher, 1937; Ajamil, Romeu, Vega & Montejo (1940).

More recently, Ruiz Rivas (1950) has found another way of emphasizing the contrast between the kidney and its surroundings by means of insufflation. Pure oxygen is introduced by means of a needle inserted between the tip of the coccyx and the posterior anal margin. The gas ascends as a subserous emphysema through the pelvic cavity into the thoracic cavity and neck. Rivas writes that the renal fascia forms a cul-de-sac, 'closed in on all sides except its lower extremity where there exists an ample aperture communicating with the retroperitoneal tissue...'. When air is introduced into the pelvic fascia, the emphysema infiltrates the retroperitoneal tissue and penetrates the inferior opening of the whole perirenal space so that a perirenal emphysema occurs on both sides.

According to this description the fascial envelope of one kidney communicates with that of the other below the level of the lower poles of the kidneys.

Blackwood (1951) agrees with this view, and suggests that the truth about the disposition of the renal fascia is midway between the old classical conception and the new one suggested by Mitchell. He writes that 'air readily tracks up the retroperitoneal tissues from the presacral area and the perirenal emphysema is often complete within a few minutes. When the air reaches the lower part of the kidney, it usually diffuses in an even manner over its anterior and posterior surfaces. There does not appear to be any anatomical barrier to this even perirenal diffusion.' He goes on to say that 'in most cases in which the air has been injected on one side only, there has been no tendency for it to cross the midline once it has ascended to the

renal areas. Also, in only one instance has air tracked superiorly to reach the thorax, and then in only a small amount. The clarity with which the suprarenal glands are usually demonstrated confirms the belief that the perirenal fascia invests both the kidney and the suprarenal. However, the ease with which injected air will fill the perirenal space from below would support the view that the space is in effect an open one inferiorly.'

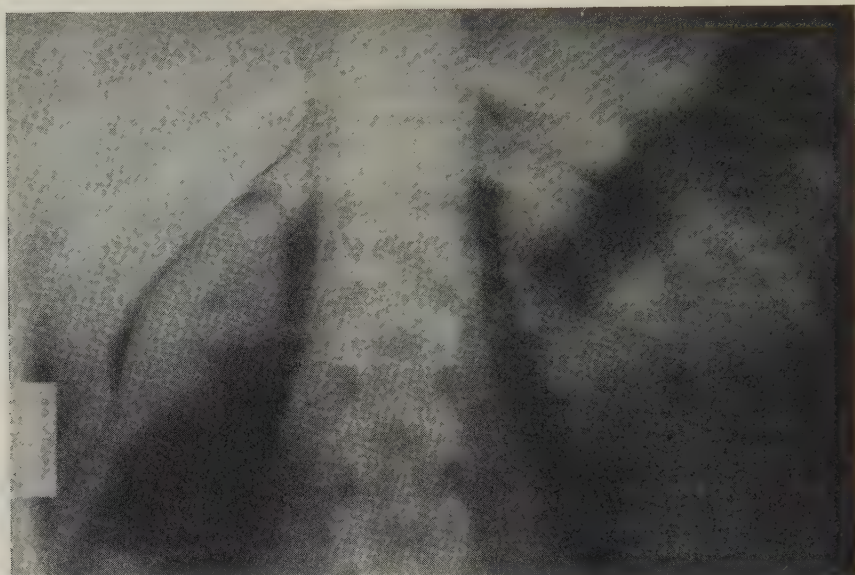


Fig. 1. X-ray view (laminagraph) of kidneys and suprarenal glands after presacral insufflation by Rivas's method.

Tinckler (1953) agrees with this view, which is also confirmed by my own observations. When gas is injected in the midline by Rivas's method, it can be seen to streak from the midline to the fascial space round both kidneys. If it is desired to keep most of the air to one side, all that is necessary is to elevate that side. If one wishes to fill both sides by a single injection, this can be accomplished in several ways: one half of the gas can be injected with the left side of the body higher than the right, and the remainder with the right side higher; alternatively, the gas can be injected with the patient prone and then equalized by placing the side with the least amount of gas uppermost (see also Steinbach, 1952). Massage sometimes helps to get the air across the midline, as Steinbach (personal communication) has also observed.

Radiological observations based on Rivas's method thus imply that there is a continuous fascial space from the point of injection to the space around the kidneys, and that the perirenal spaces are therefore open below. The possibility that the perirenal spaces also communicate across the midline is suggested by the earlier observation of Mencher (1937) and of Ajamil *et al.* (1940). I have not myself observed that gas which is introduced by Rivas's method can flow from one fascial space to the other at the level of the kidneys themselves.

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## A CASE OF HERNIA INTO THE DESCENDING MESOCOLON

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The above condition was found in a dissecting-room subject, a man of 78 years, whose death was due to arteriosclerosis.

The small intestine was entirely hidden (Fig. 1) by an abnormal sheet of peritoneum which was attached at its periphery to the ascending, descending and pelvic parts of the colon, and to the posterior abdominal wall along the root of the transverse mesocolon.

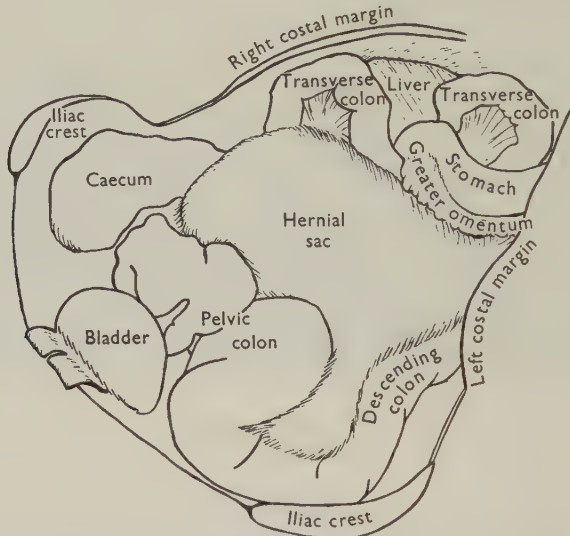


Fig. 1. The relative positions of the hernial sac and the abdominal viscera.

Dissection also revealed an abnormal course of the left colic artery and the inferior mesenteric vein. Instead of both running upwards between the root of the mesentery and the descending colon, the left colic artery (Fig. 3A) turned downwards and medially in front of the inferior mesenteric artery, over the common iliac arteries and then over the ileum as it disappeared behind the peritoneum three inches above the ilio-caecal junction; here it was joined by the inferior mesenteric vein which, after emerging from the pelvis, swung over to the right side crossing the same structures as the left colic artery. The two vessels ran upwards close to the right of the root of the mesentery, crossed the superior mesenteric vessels and parted company 1 in. below the pancreas. The vein, joined by a tributary from the splenic flexure, continued upwards deep to the neck of the pancreas to the junction of the splenic and superior mesenteric veins. The artery, joined by a branch from the middle colic artery, turned sharply in the direction of the splenic flexure and could be traced

as a discrete vessel alongside the descending colon as far as the iliac fossa where it anastomosed with another branch of the inferior mesenteric artery.

Many writers, notably Moynihan (1906) have followed the example of Treitz (1857) and called this abnormality a para-duodenal hernia, herniation of small intestine into the para-duodenal fossa. But Andrews (1923) and Callander, Rusk & Nemir (1935) contended that duodenal herniae were really congenital abnormalities, adding in support of their theory that there is no differential pressure within the abdomen to push small intestine into one of these fossae, and there is never omentum within the sac (the commonest content of herniae elsewhere). Although theirs is a more convincing argument, recent writers still

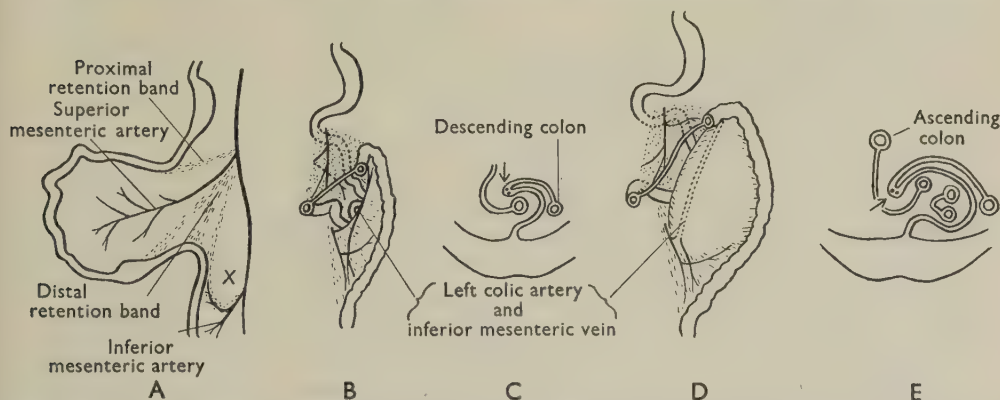


Fig. 2. A, the proximal and distal retention bands (after Frazer & Robbins (1915)). (X) indicates the part of the mesocolon which meets the first loop of returning small intestine. B-E, steps in the development of a hernia into the descending mesocolon. The arrows indicate the left colic artery and inferior mesenteric vein.

adhere to the older theory, e.g. Aird (1949) writes 'The defect of Andrew's theory is that it cannot easily be applied to the para-duodenal hernia of Landzert...' This attitude is not justified, however, because the congenital abnormality theory is strengthened by using the following feature in the normal process of the rotation of the intestine described by Frazer & Robbins (1915). These authors noted the presence of two retention bands—condensations of mesenchyme—in the dorsal mesentery, preventing the bowel above the duodeno-jejunal flexure and below the splenic flexure from entering the umbilical cord (Fig. 2A). The colic or distal retention band spreads out like a fan on reaching the splenic flexure and further, the lowest part of the fan sweeps downwards and backwards to the posterior abdominal wall along the left colic artery.

If this retention band, and especially the lower sweep, were to outlive its usefulness and remain resistant while small intestine is returning to the abdomen, obviously (Fig. 2B-E) the descending mesocolon above the left colic artery will yield more readily and form a sac lying first behind the remainder of the mesocolon and later protruding between the artery and colon; Fig. 3A, although primarily for the course of the vessels, and Fig. 3B, demonstrate how the two vessels become thrown over the whole of the small intestine and how the neck of the sac comes to face and fuse with the posterior abdominal wall.

Another abnormality in this subject, but of no significance as far as the hernia is concerned, involved the transverse colon, a loop of which has passed up behind the stomach and appeared above the lesser curvature covered with lesser omentum (Fig. 1).

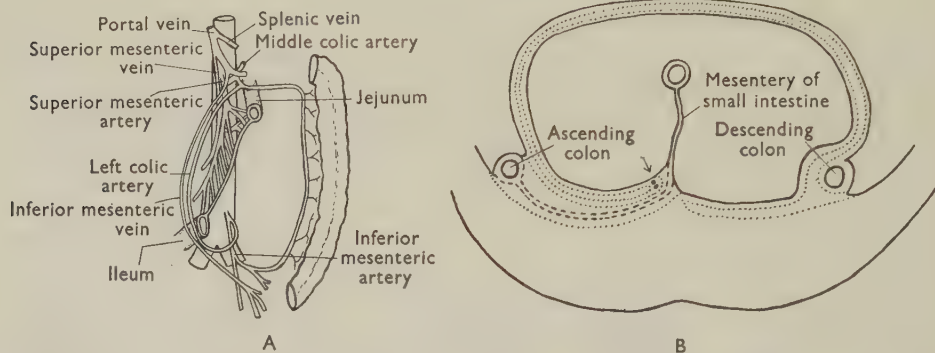


Fig. 3. A, the abnormal course of the left colic artery and inferior mesenteric vein. B, the final position of the mesenteries in a cross-section through the hernia. The obliterated layers of peritoneum are represented by dotted or broken lines. The left colic artery and inferior mesenteric vein are indicated by the arrow.

#### SUMMARY

A case of hernia into the descending mesocolon involving most of the small intestine is described. Although not accepted by Aird (1949) the theory advanced by Andrews (1923) and by Callander *et al.* (1935) that the condition is an abnormality of development is supported but with some modifications.

I am indebted to Prof. R. D. Lockhart and Mr G. L. Purser for their helpful advice and criticism in the preparation of this paper.

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# THE ORIGIN AND FATE OF THE URETHRAL PLATE IN MAN

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Apart from Barnstein & Mossman (1938) investigators of this problem agree that the urethral plate is an outgrowth from the walls of the cloaca and of the urogenital sinus.

Some workers (Debière, 1883; Tourneux, 1889; Schwartztrauber, 1904; Lichtenberg, 1906) consider that the region from which the urethral plate develops is of ectodermal origin, and consequently the urethral plate and the whole penile urethra are derived from ectoderm. Others (Herzog, 1904; Felix, 1912; Johnson, 1920; Williams, 1952) believe that the urogenital sinus is an endodermal derivative. Consequently according to these authors, the urethral plate and the resulting glandar urethra are of endodermal origin, the remainder of the penile urethra developing from the urogenital sinus itself.

Van den Broeck (1909) agrees that the urethral plate is of endodermal origin, but considers that it contributes to the lining of all parts of the penile urethra, and that surface epithelium covering the urethral folds is incorporated into the floor of the urethra.

Siddiqi (1937) considers that the dorsal half of the urethral plate develops from the urogenital sinus, whereas the ventral part is derived from the ectodermal cloaca. Thus the bulbo-urethral glands, the bulb, the floor of the proximal two-thirds and the whole of the distal third of the urethra are ectodermal, but the roof of the proximal two-thirds of the urethra is purely endodermal in origin.

Berry Hart (1908) and Wood Jones (1910, 1914) suggest that the glandar urethra results from the canalization of an ectodermal cord of cells which joins the lumen of the spongy urethra, itself derived from endodermal tissue.

Barnstein & Mossman (1938) have enunciated a completely different concept. According to them, the urethral plate develops as an ectodermal ingrowth from the urethral groove along the under surface of the phallus. This ectodermal lamina gives rise to the whole penile urethra and the bulbo-urethral glands.

## AIM OF THE INVESTIGATION AND MATERIAL

In order to ascertain whether the conclusions of Barnstein & Mossman (1938), based on observations made on squirrel embryos, could be applied to man, a series of thirty-seven human foetuses (twenty-one male, twelve female and four in the indifferent stage) have been specially serially sectioned either in the coronal or in the sagittal plane. The length of the specimens varied from 10 to 150 mm. (crown-rump).

The histological findings in the phallic region have been correlated with those in the gonad and with the appearances of the external genitalia. The stage of differentiation of the foetal gonads has been assessed by applying the criteria of Gillman

(1948). When describing the foetuses it is assumed that the foetus is in the upright posture with the phallus pointing forwards. Thus the tip of the phallus is anterior, the base posterior and the surface which is continuous with the infra-umbilical abdominal wall is superior, whereas that in continuity with the perineum is inferior.

## OBSERVATIONS

### *Indifferent embryos*

*In the 10 mm. embryo* neither genital tubercle nor genital swellings are present and the gonad consists of thickened germinal epithelium. The part of the anterior wall of the cloaca which adjoins the cloacal membrane is thickened (Pl. 1, fig. 1) to form the incipient urethral plate which appears to be quite distinct from the surface epithelium adjoining and partaking in the formation of the cloacal membrane.

*In the 12 mm. and the 13 mm. embryo* the genital tubercle has appeared, though there are no genital swellings. The gonadal primordia consist of thickened coelomic epithelium from which short sex cords extend into the underlying mesenchyme. The urethral plate consists of a short lamella extending into the genital tubercle from the fused converging walls of the anterior portion of the cloaca and primitive urogenital sinus (Pl. 1, fig. 2). The lower margin of this lamella is in contact with the surface epithelium but is quite distinct from it. It is noted that the cytoplasm of surface epithelial cells in contact with presumed endodermal cloacal tissue of the urethral plate contains refractile, eosinophilic granules. The mesenchyme on either side of the urethral plate is proliferating to raise low folds, covered by surface epithelium and disposed along the long axis of the tubercle on either side of the midline. These urethral folds, and the enclosed urethral groove, do not however reach the tip of the phallus, where the surface epithelium, in relation to the anterior extremity of the urethral plate, proliferates.

*In the 16 mm. embryo* the genital tubercle consists of a conical structure 1 mm. long and possessing a well-marked urethral groove along its under-surface. Genital swellings are recognizable in relation to its base. The gonad is still indifferent. The cloacal region is almost completely separated by the urorectal septum and the urogenital sinus is subdivided into pelvic and phallic portions. The urethral plate extends from the converging walls of the phallic portion of the urogenital sinus to the tip of the phallus where the surface epithelium reacts to the contact by proliferating. The surface epithelium in relation to the posterior portion of the plate is however showing signs of retrogression.

### *Male foetuses*

*In the 18 mm. foetus* the genital tubercle is 0.8 mm. long and the gonad is showing signs of differentiating into a testis. The definitive urogenital sinus is completely separate from the rectum and the primitive urogenital ostium has been established. Sagittal sections through the genital tubercle show clearly that the urethral plate is a cellular proliferation continuous with the anterior wall of the phallic portion of the urogenital sinus and extending to the tip of the genital tubercle (Pl. 1, fig. 3). In this region the reactive proliferation of the surface epithelium has begun to form a tag which is obvious under the microscope though not to the naked eye.

*In two 20 mm. foetuses* the genital tubercle is 1 mm. long and bears a well-marked urethral groove extending to near its tip. The terminal tag can be identified macroscopically and the genital swellings are prominent. The gonads in both cases present the characteristics of early testes. The urethral plate, folds and groove present no new features (Pl. 1, figs. 4, 5).

*In the 24 mm. foetus* the genital tubercle has become converted into a cylindrical phallus. It is 1.2 mm. long and has a distinct curvature in a caudal direction. The well-developed genital swellings are situated on either side of the base of the phallus. The gonads are early testes in which the four zones described by Gillman (1948) are readily recognizable. Although the urethral plate has the shape of a lamella throughout most of its length, near the base of the phallus where the surface epithelium in relation to the plate is showing signs of retrogression, the lower, more superficial part of the plate is slightly thickened.

*The 25 and 26 mm. foetuses* are essentially similar to one another but they differ from the 24 mm. foetus in that the end of the phallus is globular and a coronary sulcus has appeared. The curvature of the phallus, which is about 1.5 mm. long, is more marked in the 25 mm. specimen than in the 26 mm. one. The gonads present no new features. In the phallic portion of the urogenital sinus near its junction with the pelvic portion, cellular outgrowths into the surrounding mesenchyme have developed from the lining epithelium. These cellular buds are the primordia of the bulbo-urethral glands. In the phallic region it is noted that the urethral plate traverses and reaches the tip of the newly defined glans. The epithelial tag is the surface indication of the anterior extremity of the urethral plate.

*In the 30 mm. foetus* the phallus is 2 mm. long, slightly curved and bears a groove which extends to the base of the glans of the future penis. The gonads are differentiating into testes in which the interstitial cells are more abundant, better defined and beginning to enlarge. At, or near, the tip of the phallus, the surface epithelium, in relation to the anterior extremity of the urethral plate, reacts by producing a marked proliferation—the terminal tag. As the surface epithelium in relation to the lower margin of the urethral plate is traced towards the base of the phallus the character of the epithelium gradually changes from one of proliferation to one of thinning out and retrogression. The lower (superficial) third of the urethral plate is thickened where it lies in contact with this retrogressing epithelium lining the roof of the urethral groove (Pl. 1, fig. 6).

*Twin 34 mm. foetuses* possess phalluses 2 mm. long which are distinctly curved. The gonads are testes in which the interstitial tissue is abundant and contains several large, pale, eosinophilic cells. Sections through the phallus show that the urethral plate traverses the glans and reaches its tip (Pl. 1, fig. 7) where the surface epithelium still reacts by proliferating. Near the base of the phallus, however, the thickened part of the urethral plate is disintegrating (Pl. 1, fig. 8), and leaving epithelial bridges across the resulting groove which deepens the furrow formed by the outgrowth of the urethral folds.

*In the 45 mm. foetus* the phallus is 2 mm. long, markedly curved caudalwards and bears a large tag. The interstitial cells of the developing testis are numerous, large and their eosinophilic cytoplasm has a granular appearance. Owing to the obliquity of the sagittal plane of section through the phallus the following facts are revealed:



the urethral folds are approaching one another in relation to the posterior portion of the phallic portion of the urogenital sinus; the lower (superficial) part of the urethral plate has become thickened well caudal to the coronary sulcus; caudal to this region in which the urethral plate is thickened, disintegration of the thickened portion of the plate deepens the shallow groove previously formed by the outgrowth of the urethral folds (Pl. 2, fig. 11); the urethral plate is still lamellar in section throughout its course in the glans.

*In the 51 mm. foetus* the phallus is 3.5 mm. long and only slightly curved. The urethral folds have fused, so bringing the urogenital ostium just proximal to the coronary sulcus. The fusion of the folds has resulted in the formation of a perineal raphé which extends from the anus to the urogenital ostium. The now rounded genital swellings appear to have migrated towards the anus and no longer flank the base of the phallus. In the developing testes the interstitial cells are highly developed and widely separate the sex cords. In the phallic region closure of the urethral folds has taken place, and is still taking place, in such a way that fusion of the portion of these folds in relation to the phallic part of the urogenital sinus has given rise to the bulb of the urethra; while closure of the portion of the folds flanking the part of the urethral groove derived from the urethral plate, gives rise to the spongy urethra distal to the bulb. Closure of the phallic part of the urogenital sinus and of the urethral groove takes place in such a way that only epithelium derived from the sinus or from the urethral plate is included in the lining of the urethra in this region. Surface epithelium is completely excluded.

In the region of the urogenital ostium and anterior to it, the urethral plate is deepening the urethral groove in the same way as was described along the shaft of the phallus of previous specimens. An infolding or ingrowth of surface epithelium to meet the anterior extremity of the urethral plate has appeared. Associated with this ingrowth at the tip of the glans, the urethral folds appear to be relatively more highly developed on the glans than they were along the shaft of the phallus in earlier stages.

*In the 53 mm. foetus* the features were similar to those found in the 51 mm. foetus. However, examination of the sections through the phallic region show that closure of the urethral groove has taken place in such a way that, not only is all surface epithelium excluded, but some of the epithelium derived from the urethral plate has been 'locked out' and left on the surface to be included in the perineal raphé (Pl. 2, figs. 12, 13). Once fusion of the urethral folds has taken place, the resulting urethra becomes separated from the surface by mesenchymal tissue and comes to lie deeper in the substance of what may now be called the foetal penis.

*In the 57 mm. foetus* the urogenital ostium is encroaching on the glans and the preputial folds have begun to develop. There is still a considerable tag at the summit of the penis.

*In the 65 mm. foetus*, in addition to these features, the scrotum forms a single eminence at the root of the free portion of the penis, and *in the 70 mm. foetus* the urogenital ostium is confined to the under-surface of the glans which is half covered by the developing prepuce.

Sections through the penis of these three foetuses reveal similar characteristics: The urethral plate is present in the roof of the distal part of the formed spongy

urethra and extends to the tip of the glans, where it comes in contact with the ingrowth of surface epithelium. In the region where the two types of epithelium contact one another they react by proliferating and thickening (Pl. 2, fig. 14). Further back these thickenings disintegrate to give rise to the urethral groove in relation to the urogenital ostium. This groove on the glans penis is wider than that found along the shaft of the phallus in earlier stages (Pl. 2, fig. 15). In the caudal part of the groove the lining epithelium is derived entirely from the reactive proliferation of the urethral plate (Pl. 2, figs. 16, 17). In the 70 mm. foetus the groove has closed to form the proximal part of the glandar urethra in such a way that only epithelium derived from the urethral plate is included in the lining (Pl. 2, fig. 18). Cellular buds, growing out from the epithelium lining the roof of the proximal part of the penile urethra, have been observed in all three specimens.

A noteworthy feature observed in these foetuses is that when the proliferation derived from surface epithelium disintegrates it produces 'epithelial pearls' similar to those found in the preputial 'glandar lamella' as opposed to the formation of epithelial bridges which occurs in urethral plate proliferations (cf. Pl. 2, fig. 14, and Pl. 3, fig. 19, with Pl. 1, figs. 8, 9).

*In the 80 mm. male foetus* the glans which still bears a tag is almost completely invested by the prepuce. The urogenital ostium is approaching the tip of the glans. Sections through this region show that the ingrowing surface epithelium is responsible for most of the reactive proliferation at the anterior extremity of the urethral plate which is now relatively poorly developed and contributing little to the reactive proliferation (Pl. 3, fig. 19). Disintegration of the reactive proliferation gives rise to a groove lined entirely by epithelium of surface origin. Closure of this groove gives rise to a terminal portion of the glandular urethra lined by cells of ectodermal origin (Pl. 3, fig. 20).

*The 90 mm. male foetus* presents features very similar to those noted in the 80 mm. male foetus, but in *the 100 mm. male foetus* the urinary meatus appears to have been established at the tip of the glans penis. Sections through the spongy urethra reveal many cellular buds (the rudiments of the glands of Littre) extending from the epithelium lining the urethra, especially its roof and walls. In the proximal part of the glandar urethra, epithelial buds are confined to the roof. As the urethra is traced through the glans, its lumen is seen to be compressed from side to side, and a poorly developed urethral plate can still be identified in this part of the urethral roof (Pl. 3, fig. 24). The urethral plate, however, appears to be spent, and no longer reacts to the contact with surface cells. In the distal part of the glans, the urethra reverts to a more circular outline in section. This terminal portion of the urethra is derived from the thicker, lower portion of the ingrowth of surface epithelium, which has grown to meet the urethral plate (Pl. 3, figs. 21-23). The upper part of this ingrowth gives rise to a lamella, which is distal to, and quite distinct from, the urethral plate (cf. Pl. 3, fig. 23, with Pl. 3, fig. 24). At the tip of the penis the urethral folds are growing towards one another to form the most distal part of the urethra (Pl. 3, figs. 21, 22).

*The 115 mm. male foetus* still possesses what appears to be a reactive epithelial tag at the summit of the glans, otherwise the external genitalia appear fully formed. The urethral plate is no longer identifiable in this specimen, and its former site is

indicated in the proximal part of the glandar urethra by cellular buds extending from the roof of the latter. The urinary meatus has been established at the summit of the glans, and what appears to be a tag at the tip is no longer a reactive proliferation at the junction of the urethral plate and surface epithelium, but consists of a mass of epithelial cells and debris derived from the surface of the glans and the prepuce.

*In the 135 and 150 mm. male fetuses* the external genitalia are fully formed. The testes consist mainly of very coiled tubules, the interstitial cells having dwindled in number and size. Examination of sections through the penis reveal that in addition to rudiments for Littre's urethral glands there are some hollow rudiments for minor lacunae in the roof of the proximal glandar urethra and most distal spongy urethra, at the site formerly occupied by the urethral plate. No cellular buds are seen extending from the distal glandar urethra whose lining is derived from surface epithelium. The dorsal (upper) portion of the surface ingrowth which was lamellar in the two previous stages has become thickened and by acquiring a lumen forms the lacuna magna or sinus of Guérin (Pl. 3, figs. 25-27).

#### *Female fetuses*

*In the 28 mm. fetus* the cylindrical phallus is 1.8 mm. long and is less curved than in the 24 mm. or the 25 mm. male stage. The urethral groove extends to the base of the glans which bears an epithelial tag near its summit. The genital swellings flank the base of the phallus. The gonad is differentiating into an ovary.

The phallic portion of the urogenital sinus, the bulbo-urethral (greater vestibular) gland rudiments, the urethral plate and the urethral folds present features which are essentially the same and as well-defined as those found in the 24, 25 and 26 mm. male fetuses.

*In the 32 mm. fetus* the phallus is 2 mm. long, not curved and has a distinct coronary sulcus. The epithelial tag and urethral groove are as well-developed and as extensive as in the 30 mm. male fetus. The gonads consist of ovaries in the growth phase (Gillman, 1948). Sections through the phallic region show the same features as were observed in the 30 mm. male fetus.

*In a second 32 mm. female fetus* the only difference was that the phallus was markedly curved in a caudal direction, and though a terminal epithelial tag could be identified microscopically, it was invisible to the naked eye.

*In two 37 mm. fetuses* the phallus is 2.2 mm. long, only slightly curved, and bears a well-developed epithelial tag. The coronary sulcus is very clearly defined and the urethral groove appears to stop short of it. The gonads present the features of ovaries in the growth phase. Sections through the shaft of the phallus show that, in female fetuses, the urethral groove is also derived from two sources (Pl. 1, fig. 9). The superficial portion results from the outgrowth of urethral folds covered by surface epithelium; the deep portion of the groove results from the breaking down of the thickened portion of the urethral plate. The urethral groove is therefore formed in female fetuses by processes which are identical with those taking place in male fetuses.

Although to the naked eye, the urethral groove appears to stop short of the coronary sulcus, microscopic examination shows clearly that the urethral folds do in fact extend on to the under-surface of the glans in both fetuses.



*In the 40 mm. foetus* the phallus is 2.2 mm. long and only slightly curved. The urethral groove appears to reach the base of the glans and the epithelial tag is small. The genital swellings still flank the base of the phallus. The gonads are ovaries in the growth phase similar to those found in the 37 mm. fetuses. The urethral groove and plate present the same features as in the 34 mm. male and 37 mm. female fetuses.

It should be noted that the process of disintegration in the thickened portion of the urethral plate to deepen the urethral groove starts well behind the coronary sulcus, and the resulting furrow is as extensive in the female as it is in the male.

This specimen presents one outstanding feature, namely the slight degree of reaction in the epithelium in relation to the anterior extremity of the urethral plate. Hence the smallness of the terminal tag.

*The 42 mm. foetus* possesses a phallus 2 mm. long, distinctively curved caudalwards and bearing a well-developed epithelial tag. The urethral groove extends to the coronary sulcus. The foetal ovaries are in the growth phase.

Owing to the curvature of the phallus the sections (coronal plane of the embryo) through the tip of the phallus are longitudinal and serve to show that the urethral plate reaches the tip of the organ as well in the female as in the male fetuses even at this comparatively late stage (Pl. 2, fig. 10).

*The 44 mm. foetus* was sectioned in the sagittal plane and presented the same features as were observed in the 42 mm. foetus.

*The 50 mm. foetus* has a phallus 2.8 mm. long which is less curved than that of the 42 and 44 mm. female or the 45 mm. male fetuses. The terminal tag is but poorly developed and the urethral groove is visible only along the proximal three-quarters of the shaft of the phallus. On microscopic examination it is seen that the ovaries are in the growth phase. The urethral groove extends on to the under surface of the glans, while in relation to the anterior extremity of the urethral plate there appears to be either an infolding or an ingrowth of surface epithelium. This ingrowth is comparable to that seen in the 51 mm. male foetus.

*In the 55 mm. foetus* the external genitalia are so disposed that it is possible to diagnose the sex of the foetus from their examination. The phallus, though it still bears a tag, is markedly bent in a caudal direction. The genital swellings are elongated and still flank the base of the phallus. There is a distinct groove in the perineum and along the proximal three-quarters of the phallus: there is no perineal raphé. Although the external genitalia have assumed a distinctly female character, the ovaries are still in the growth phase and present histological features which are similar to those found in earlier stages.

The urethral groove as well as remaining open is wider and flatter than in earlier female fetuses. Near the tip of the phallus the meeting of the infolding or ingrowing surface epithelium and the anterior extremity of the urethral plate results in both types of epithelium proliferating and becoming thickened.

*In the 80 mm. female foetus* the remains of the urethral plate can still be identified in the glans, and there is still a reactive proliferation and thickening where it meets the surface ingrowth of epithelium. This proliferation shows little sign of disintegration; the urethral groove is very wide and flattened on the under-surface of the clitoris.

In the 100 mm. foetus the external genitalia are clearly female in character, and the groove between the labia minora is continued on to the proximal part of the glans clitoridis. The urethral plate can no longer be identified in this specimen.

#### CONCLUSIONS AND DISCUSSION

The examination of the external genitalia of this series of foetuses and the correlation of the findings with the histology of their respective gonads has confirmed the opinion of Wilson (1926) that Spaulding's criteria (1921) for the diagnosis of sex in foetuses between the 25 and 50 mm. stage are most unreliable. The 50 mm. stage is the earliest stage at which sex can be determined from the external appearances of the genitalia, with any degree of certainty.

In considering the origin of the urethral plate it may be stated that it is an outgrowth from the walls of the cloaca and of the urogenital sinus. The development of the urethral plate starts at the 10 mm. stage when it is recognizable as a thickening in the anterior wall of the endodermal cloaca. No evidence has been found in the embryos constituting the present series to support the statement of Barnstein & Mossman (1938) that the urethral plate is an ectodermal ingrowth from the urethral groove along the under surface of the phallus. However, when considering from which germ-layer the urethral plate is ultimately derived, the participation of ectodermal tissue in its formation cannot be excluded. Zuckerman (1940) pointed out that the floor of the urogenital sinus is the ventral part of the cloacal membrane, which incorporates, in its development, the primitive streak. It is in this region of the early embryo that cells stream in from the surface to deeper structures, and the possibility exists that its potency and plasticity are not completely lost in later stages of development. Thus the inclusion of ectodermal cells in the lining of structures from which the urethral plate develops may account for the results of workers such as Burns (1942, 1945 *a, b*) who have investigated the effect of sex hormones on the urogenital sinus and its derivatives.

As regards the formation of the penile urethral in man the following new concept is presented:

The urethral plate grows forward to reach the tip of the phallus in foetuses of both sexes. Consequently, the tissues are relatively less well differentiated at the tip of the phallus than they are at the base, and it is reasonable to assume that the contact of the urethral plate with the surface epithelium is more recent at the tip of the phallus than at the base. Initially, surface epithelium reacts to contact with the urethral plate by proliferating to form a terminal epithelial tag which becomes the surface indication of the anterior extremity of the urethral plate. This initial stimulation is followed by a retrogression of the surface epithelium, a phenomenon that is observed further back along the shaft of the phallus. The urethral plate having thus 'overcome' the surface epithelium now thickens in its lower, superficial portion, where it is in contact with the retrogressing epithelium.

While these changes take place in foetuses of either sex, a urethral groove is established by the development of urethral folds along the under-surface of the phallus on either side of the urethral plate and phallic portion of the urogenital sinus. These folds are covered by surface epithelium, and it is suggested that the groove between them be called the *primary or primitive urethral groove*. A *secondary*

*urethral groove* develops at the 35 mm. stage as a result of the disintegration of the thickened portion of the urethral plate which lies in the roof of the primary groove. The secondary groove thus deepens the portion of the primary one anterior to the open phallic portion of the urogenital sinus and the *definitive urethral groove* is established. In this way two types of epithelium derived from different sources line the groove, and the junction of the epithelia is situated on the inner aspect of the resulting folds. Up to about the 50 mm. stage male and female foetuses develop along identical lines. In female foetuses the urethral folds give rise to the labia minora, and thus the junction between surface epithelium and epithelium ultimately derived from the urogenital sinus is not, as is often stated, at the free margin of the labia minora but on their inner aspect. This observation fits in with the histological findings in the adult labia which are covered on both surfaces with skin, the transition to mucous membrane being on the inner aspect of the labia at or near the base.

The common misconception that the urethral plate is but poorly developed in female foetuses, or that it does not encroach on the glans in foetuses of either sex must also be dispelled. In male foetuses at about the 50 mm. stage when the interstitial cells of the testis have become numerous, large, eosinophilic, granular and therefore possibly functional, the urethral folds start to grow together and fuse to form the bulb and spongy portions of the penile urethra. The fusion also results in the formation of the perineal raphé and starts opposite the most posterior part of the phallic portion of the urogenital sinus and proceeds thence towards the glans. The bulb of the urethra is formed by closure of the phallic portion of the urogenital sinus, but the spongy urethra is formed by closure of the urethral groove. The urethral folds grow together and fuse in such a way that only epithelium lining the urogenital sinus, or the secondary urethral groove, is included in the lining of the urethra.

From this stage hence, the diagnosis of the sex of the foetus presents no difficulty, as no fusion of the folds occurs in the female. In addition, the genital swellings remain separate, elongated and continue to flank the base of the phallus, whereas in the male the genital swellings become rounded, migrate caudally, then fuse to form a single scrotum which comes to lie caudal to the base of the penis.

At about the 60 mm. stage the proximal portion of the glandular urethra begins to be formed by processes which are similar to those responsible for the formation of the spongy urethra, and the lining of the proximal part of the glandular urethra is thus derived from the urethral plate.

In the formation of the distal part of the glandular urethra three additional factors need to be considered:

In the first place the urethral folds appear to be relatively more highly developed and to grow together, and fuse in such a way that surface epithelium which reacts to contact with the urethral plate by proliferating and breaking down comes to be incorporated in the lining of the urethra.

Secondly, the urethral plate appears to be spent and dwindling when the terminal portion of the urethra differentiates. By the 115 mm. stage the urethral plate has ceased to exist as such.

Thirdly, there is a lamellar ingrowth of surface epithelium which grows towards



the anterior extremity of the urethral plate. This ingrowth gives rise to the lining of the fossa terminalis as far back as the valve of Guérin, while the lacuna magna or sinus of Guérin develops from a lamellar extension from the upper (dorsal or deep) part of this surface ingrowth. This observation contradicts the findings of Debière (1883), Tourneux (1889), Van den Broek (1909) and Williams (1952), who state that the lacuna magna is derived from the urethral plate. It is suggested that this lamellar ingrowth be called the lacunar plate or lamella to distinguish it from the urethral plate. Thus the explanation of the development of the distal glandar urethra would seem to be a combination of the processes described by Hunter (1935), who believed that it resulted from the fusion of folds enclosing surface epithelium, and those described by Wood Jones (1910, 1914) and Berry Hart (1908), who considered that it resulted from an ingrowth of surface cells. These authors were, however, mistaken in believing that the processes they described formed the whole of the glandar urethra.

Although the lacuna magna does not develop from the urethral plate, minor lacunae do develop from the upper (deep, dorsal) part of the urethral plate and the urethral glands of Littré (lesser vestibular glands in the female) only develop from epithelium derived either from the urethral plate or from the urogenital sinus.

The bulbo-urethral gland (greater vestibular gland in the female) rudiments do not develop as suggested by Herzog (1904) and Felix (1912) from the pelvic portion of the urogenital sinus, but they arise at the 25 mm. stage from the epithelium lining the phallic portion of the urogenital sinus, near its junction with the pelvic portion. This is before the urethral plate has given rise to the secondary urethral groove, let alone any part of the penile urethra. No evidence has been found to support the view of Barnstein & Mossman (1938) that these rudiments develop from the urethral plate with whose origin they are completely unconnected.

It is suggested that the above interpretation of the development of the urethra provides a rational basis for the understanding of congenital malformations of the external genitalia in the male, apart from epispadias, in terms of:

(a) Failure of the urethral plate and groove to develop and the phallic portion of the unrogenital sinus remaining open.

(b) Failure of the urethral groove and phallic portion of the urogenital sinus to close at all giving rise to the female type of external genitalia.

(c) Varying degrees of failure of the urethral groove to close. This group will include most cases of hypospadias, as the spongy urethral and proximal glandar urethra are developed by a similar, continuous process. The meatus may be proximal to the coronary sulcus or may extend on to the glans. The blind pocket leading into the substance of the glans from its summit which may be found in these cases results from the development of a lacunar lamella despite the maldevelopment of the more proximal portions of the urethra.

#### SUMMARY

1. Thirty-seven human fetuses (four indifferent, twenty-one male and twelve female) ranging in size from 10 to 150 mm. crown-rump length have been specially serially sectioned and examined.

2. The urethral plate is an outgrowth from the urogenital sinus.

3. The bulb of the urethra is derived from the phallic portion of the urogenital sinus.
4. The spongy urethra and proximal portion of the glandar urethra are derived from the urethral plate.
5. The distal portion of the glandar urethra is derived from surface ectoderm which also gives rise to the lacuna magna (sinus of Guérin).
6. The bulbo-urethral (greater vestibular) glands develop as outgrowths from the phallic portion of the urogenital sinus.
7. In female foetuses development of the external genitalia proceeds in a manner identical to that taking place in male embryos till about the 50 mm. stage.
8. These findings and the developmental factors involved in some congenital malformations have been correlated.

I am indebted to Prof. W. J. Hamilton for suggesting this work and for offering valuable criticism. I have been assisted by Mr R. H. Watts in the preparation of the histological material, and the photographs have been taken by Mr E. V. F. Pittock, F.R.P.S.

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## EXPLANATION OF PLATES

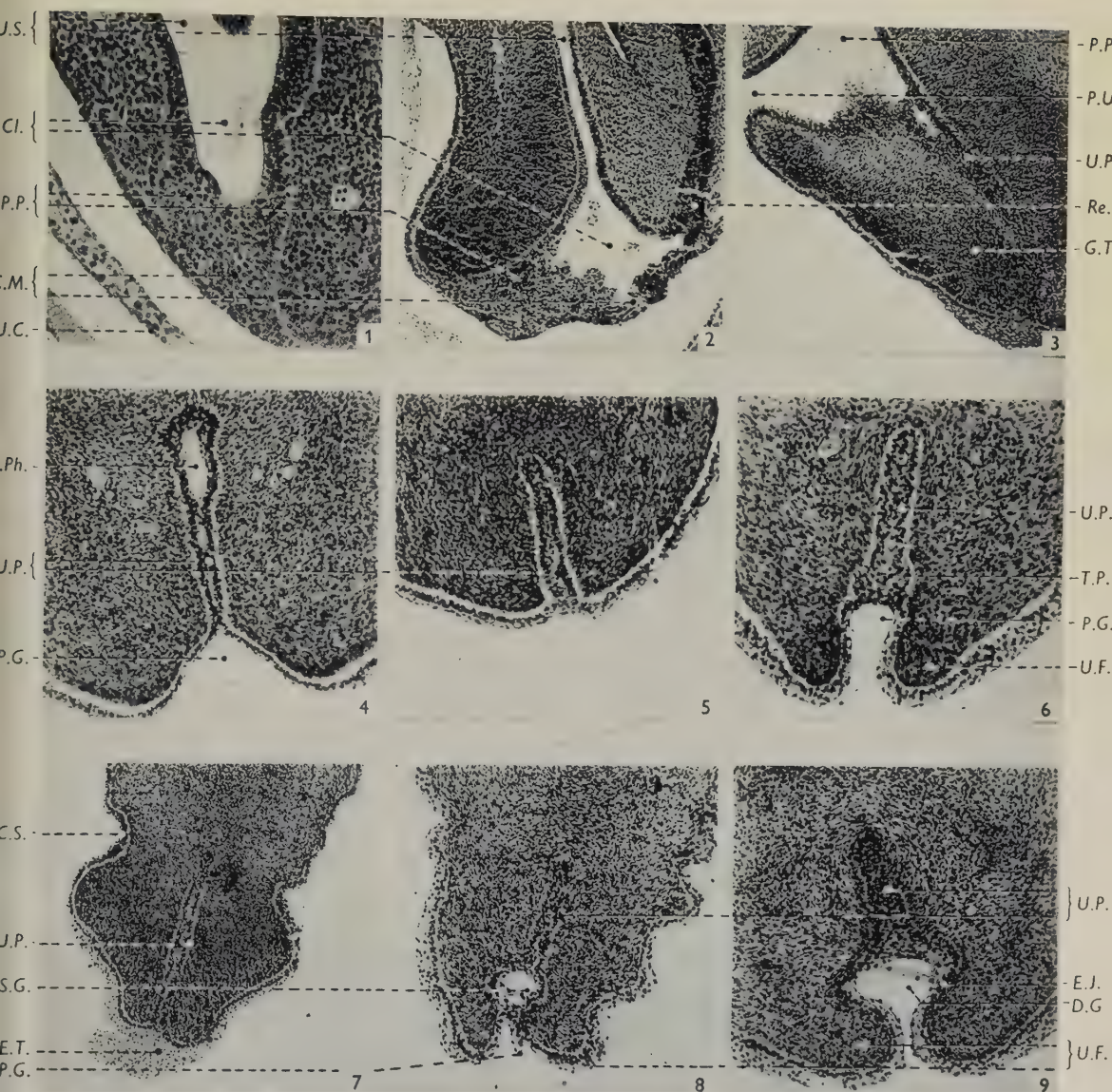
*List of Abbreviations*

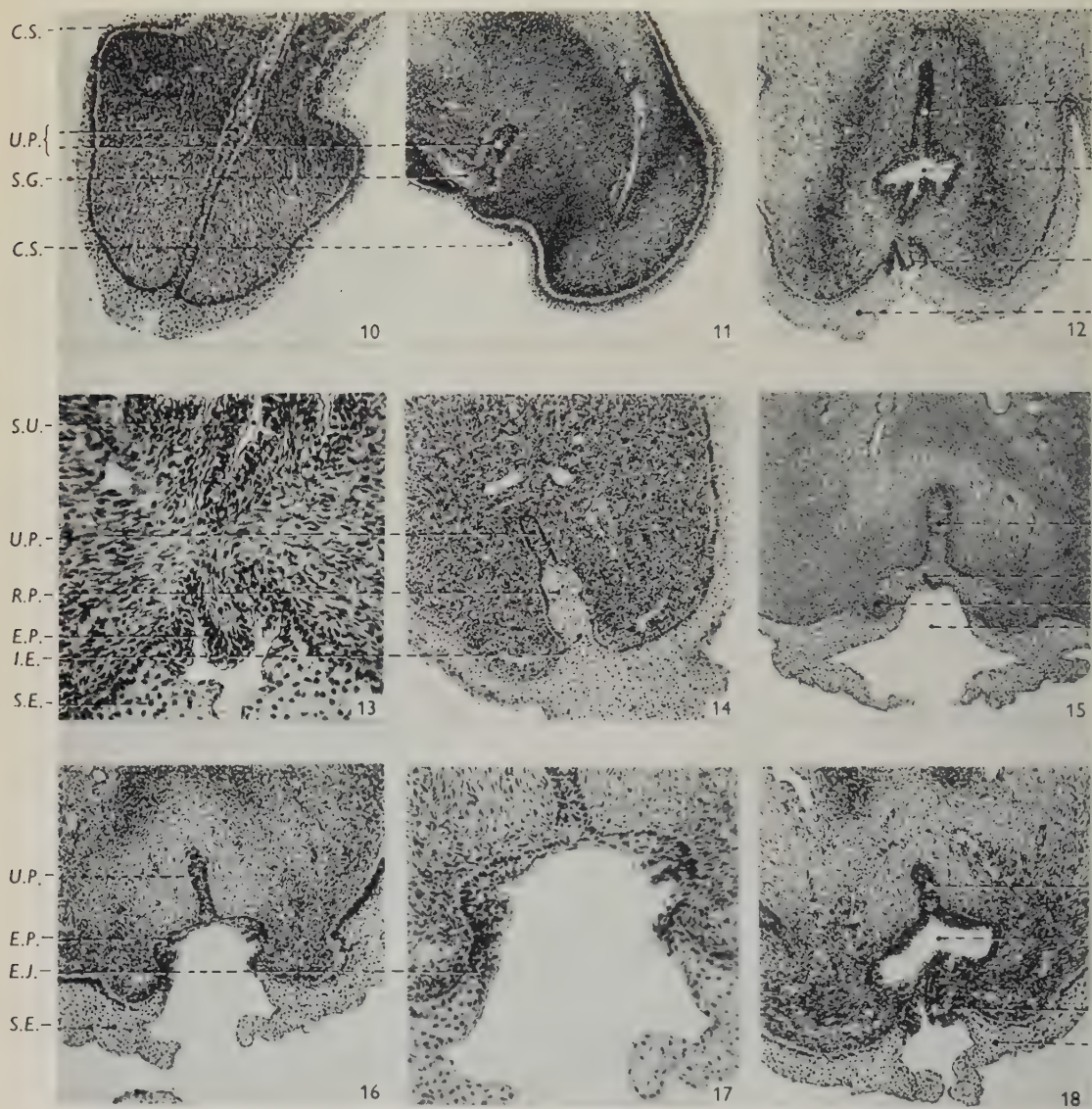
<i>Cl.</i>	cloaca	<i>L.M.</i>	lacuna magna
<i>C.M.</i>	cloacal membrane	<i>P.G.</i>	primitive urethral groove
<i>C.S.</i>	coronary sulcus	<i>P.Ph.</i>	pars phallica
<i>D.G.</i>	definitive urethral groove	<i>P.U.O.</i>	primitive urogenital ostium
<i>E.J.</i>	function of surface epithelium and epithelium derived from the urethral plate	<i>Re.</i>	rectum
<i>E.P.</i>	epithelium derived from the urethral plate	<i>R.P.</i>	reactive proliferation
<i>E.T.</i>	epithelial tag	<i>S.E.</i>	surface epithelium
<i>F.T.</i>	fossa terminalis	<i>S.G.</i>	secondary urethral groove
<i>G.L.</i>	glandular lamella	<i>S.U.</i>	spongy urethra
<i>G.T.</i>	genital tubercle	<i>T.P.</i>	thickened portion of urethral plate
<i>G.U.</i>	glandular urethra	<i>U.C.</i>	umbilical cord
<i>I.E.</i>	ingrowth of surface epithelium	<i>U.F.</i>	urethral fold
<i>L.L.</i>	lacunar lamella	<i>U.G.</i>	urethral groove
		<i>U.P.</i>	urethral plate
		<i>U.P.P.</i>	urethral plate primordium
		<i>U.S.</i>	urogenital sinus.

## PLATE 1

- Fig. 1. Sagittal sections through the cloacal region of a 10 mm. embryo, showing thickening of the anterior cloacal wall to form the urethral plate primordium.  $\times 111$ .
- Fig. 2. Sagittal section through the urogenital sinus, cloaca and genital tubercle of a 13 mm. embryo. The epithelium lining the anterior wall of the cloaca is proliferating to form the urethral plate primordium.  $\times 78$ .
- Fig. 3. Sagittal section through the genital tubercle of an 18 mm. male foetus. The primitive urogenital ostium is established and can be seen near the top left corner of the picture. The urethral plate is seen to be continuous with the anterior wall of the phallic portion of the urogenital sinus. The surface epithelium at the tip of the tubercle (bottom, right corner of picture) is proliferating.  $\times 78$ .
- Fig. 4. Transverse section through the base of the phallus of a 20 mm. foetus. The section passes through the most anterior portion of the phallic part of the urogenital sinus and shows the urethral plate being formed from the fused sinus walls. The urethral folds, flanking the primitive urethral groove, are seen in relation to the lower part of the urethral plate.  $\times 120$ .
- Fig. 5. Transverse section through the distal part of the shaft of the phallus from a 20 mm. male foetus. The urethral plate is lamellar in shape. The surface epithelium in relation to its base is considerably thicker than the epithelium at a corresponding site further back along the shaft of the phallus, cf. with fig. 4.  $\times 120$ .
- Fig. 6. Transverse section through the shaft of the phallus of a 30 mm. male foetus. The surface epithelium in the roof of the primitive urethral groove is thinned out and retrogressing. The basal portion of the urethral plate is thickened.  $\times 96$ .
- Fig. 7. Coronal, longitudinal section through the tip of the phallus of a 34 mm. male foetus. The urethral plate extends through the glans of the future penis to reach the tip, where the surface epithelium proliferates to form the epithelial tag.  $\times 60$ .









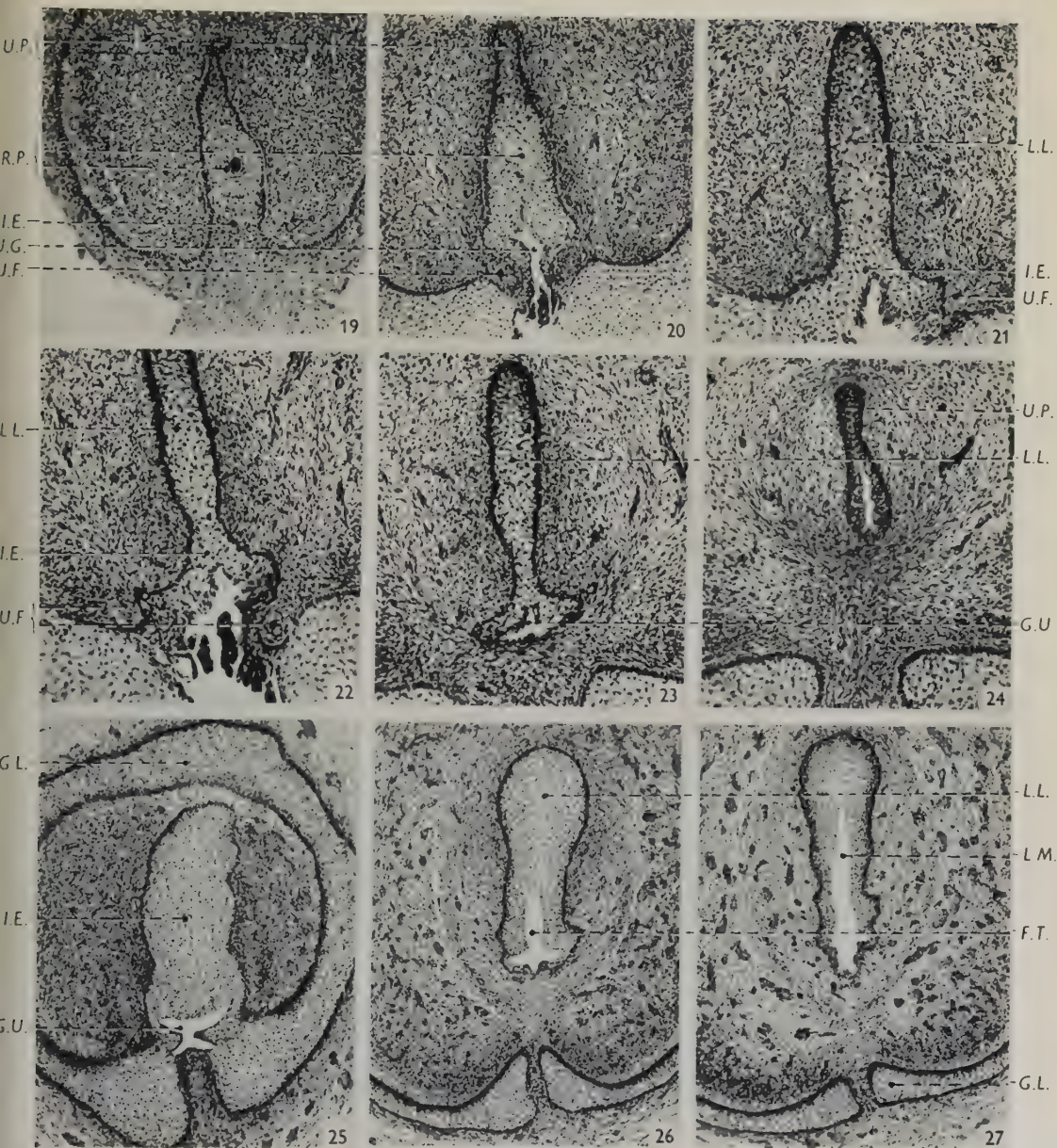






Fig. 8. Transverse section through the phallus of a 34 mm. male foetus. The thickened basal portion of the urethral plate is disintegrating to form the secondary urethral groove; epithelial bridges are seen extending across the resulting lumen.  $\times 81$ .

Fig. 9. Transverse section through the phallus of a 37 mm. female foetus. The definitive urethral groove has been established and an epithelial bridge is still present in the portion derived from the secondary urethral groove. The site of junction between the two types of epithelium lining the groove is on the inner aspect of the urethral folds.  $\times 78$ .

## PLATE 2

Fig. 10. Coronal longitudinal section through the phallus of a 42 mm. female foetus. The urethral plate is seen to extend through the glans of the future clitoris to reach the tip where the surface epithelium proliferates to form an epithelial tag.  $\times 72$ .

Fig. 11. Oblique sagittal section through the phallus of a 45 mm. male foetus. The thickened ventral part of the urethral plate is disintegrating along the shaft of the phallus, proximal to the coronary sulcus.  $\times 57$ .

Figs. 12, 13. Transverse sections through the phallus of a 53 mm. male foetus, showing that some epithelium ultimately derived from the urethral plate has been left on the surface in the course of fusion of the urethral folds. Fig. 12,  $\times 48$ ; fig. 13,  $\times 150$ .

Fig. 14. Transverse section through the tip of the penis of a 70 mm. foetus, showing surface epithelium growing in to meet the urethral plate and also the resulting reactive proliferations.  $\times 72$ .

Fig. 15. Transverse section through the glans penis of a 70 mm. foetus, showing the distal part of the urethral groove on the glans being formed from the reactive proliferation.  $\times 72$ .

Figs. 16, 17. Transverse section through the glans penis of a 70 mm. foetus, showing that the caudal part of the urethral groove on the glans is lined by epithelium derived from the urethral plate. Fig. 16,  $\times 72$ ; fig. 17,  $\times 150$ .

Fig. 18. Transverse section through the glans penis of a 70 mm. foetus, showing that closure of the urethral groove, to form the proximal part of the glandular urethra, takes place in such a way that surface epithelium is excluded from its lining.  $\times 72$ .

## PLATE 3

Fig. 19. Transverse section through the tip of the glans penis of an 80 mm. foetus. The urethral plate is poorly developed and the reactive proliferation is derived mainly from the surface ingrowth. Note the formation of an 'epithelial pearl' in the reactive proliferation.  $\times 72$ .

Fig. 20. Transverse section through the distal part of the glans penis of an 80 mm. foetus. The urethral groove is lined by epithelium derived from the surface ingrowth and the urethral folds are fusing in such a way that epithelium derived from the surface ingrowth, lines the distal portion of the glandular urethra.  $\times 72$ .

Fig. 21. Transverse section through the tip of the glans penis of a 100 mm. foetus. The ingrowth of surface epithelium has a dorsal, lamellar extension—the lacunar lamella.  $\times 102$ .

Fig. 22. Transverse section through the region of the urogenital ostium of the 100 mm. foetus, showing that the urethra is formed from the basal, thickened portion of the surface ingrowth. The epithelium lining the roof of the urethra is continuous with the lacunar lamella.  $\times 102$ .

Fig. 23. Transverse section through the distal part of the formed portion of the glandular urethra in the 100 mm. male foetus. The lacunar lamella extends from the roof of the urethra.  $\times 102$ .

Fig. 24. Transverse section through the glans penis of the 100 mm. foetus. The section passes through the part of the fossa terminalis which is derived from the urethral plate. The latter is seen as a poorly developed structure extending from the roof of the urethra.  $\times 102$ .

Fig. 25. Transverse section through the tip of the penis of a 135 mm. foetus. The surface ingrowth from which the terminal part of the urethra and the lacunar lamella are derived is seen to be continuous with the glandular lamella.  $\times 54$ .

Fig. 26. Transverse section through the terminal part of the urethra in the 135 mm. foetus. The section is taken distal to the lacuna magna and the epithelium lining the urethra and forming the lacunar lamella is identical with that of the glandular lamella.  $\times 54$ .

Fig. 27. Transverse section through the glans penis of the 135 mm. foetus at the level of the developing lacuna magna, the lumen of which is seen to extend from the urethral lumen into the lacunar plate.  $\times 54$ .

## IN MEMORIAM

LORD GEDDES. P.C., G.C.M.G., K.C.B., T.D., M.D., LL.D., F.R.S.E.

The death of Lord Geddes, in hospital at Chichester on 8 January 1954, aged 74, closes a very unusual, very distinguished career.

At the outbreak of the First World War, Auckland Campbell Geddes was Professor of Anatomy at McGill University, Montreal. His immediate return to this country as a Reserve Officer altered the whole course of his life and led to a remarkable succession of Government Offices culminating in his appointment as British Ambassador in Washington.

The story of the Geddes family and of the public services they rendered is fully told by Lord Geddes himself in a fascinating book (*The Forging of a Family*, Faber and Faber, Ltd., 1952) dictated by the author after he had been overtaken by total blindness. His father, after 25 years as a railway engineer in India moved from London to Edinburgh where his sons and daughters were educated. Auckland Campbell, born in London on 21 June 1879, was educated at George Watson's College and the University of Edinburgh, but his medical course was interrupted by service as Lieut., H.L.I., in the South African War. Graduating M.B., Ch.B., with honours in 1903 he was appointed Demonstrator of Anatomy, and in 1906, after study abroad, assistant to Professor D. J. Cunningham.

During his student days, in addition to boxing and swimming for the University and playing Rugby in the First XV, he had been an active member of the University Volunteer Corps. As Officer Commanding the University Rifle Company after his graduation he published 'A Scheme for establishing a Corps of Volunteer Officers in Reserve' (*University Review*, 1906). It is on record that Lord Haldane, who had taken office as Secretary of State for War in 1905, later acknowledged that the idea for the University O.T.C. had come to him from Auckland Geddes as also the suggestion that the 'Volunteers' should be rechristened 'The Territorial Force'.

This preoccupation with military service and the problem of National Defence was to form the background of his later career, but in the meantime Geddes pursued his professional vocation as an anatomist.

In 1909 he was appointed Professor of Anatomy at the Royal College of Surgeons of Ireland, and in 1913 left Dublin for the Chair of Anatomy at McGill University. During these years he was active in anatomical research and published a number of papers, most of them in this *Journal* between 1909 and 1913. The most notable, perhaps, were his 'Report upon an Acromegalic Skeleton' (*J. Anat.* 45, 256) based on his M.D. thesis for which he had been awarded a gold medal in 1908, 'The Origin of the Vertebrate Limb' (*J. Anat.* 46, 350) and 'The Origin of the Osteoblast and the Osteoclast' (*J. Anat.* 47, 159). He had joined the Anatomical Society at its Summer Meeting in Edinburgh in 1907 and then contributed, with the late Professor David Waterston, some notes on 'The development of the Penguin'. This formed the basis of their later joint paper 'Report on the Anatomy and Embryology of Penguins collected by the Scottish National Antarctic Expedition' (*Trans. Roy. Soc. Edinb.*, 1909).



The subsequent career of Lord Geddes from 1914 onwards has been fully covered by obituary notices in the public press; it is sufficient here to record the remarkable series of posts, military, administrative and diplomatic to which he was called through the impression he had made first on Lord Kitchener and then on Mr Lloyd George.

- 1914 Major and Second-in-Command, 17th Northumberland Fusiliers
- 1915-16 Brevet Lt.-Col., D.A.A.G., France
- 1916-17 Brigadier-General, Director of Recruiting, War Office
- 1917-18-19 Minister of National Service
- 1917-20 M.P., Basingstoke and Andover
- 1918 President, Local Government Board
- 1919 Minister of Reconstruction and President, Board of Trade
- 1919 President-elect, McGill University, Montreal
- 1920 British Ambassador, Washington

It is interesting to recall that when the Local Government Board gave place to the Ministry of Health Geddes might well have been transferred to that Department; but he continued as Minister of National Service and Reconstruction and it was another anatomist, the late Lord Addison, who was appointed first Minister of Health. It is recorded also that Geddes would have succeeded Sir Alfred Keogh as Director General of the Army Medical Service in 1917, if Lloyd George had not insisted on his replacing Mr Neville Chamberlain as Minister of National Service. A still higher office appears to have been offered him in 1919, but a serious illness prevented acceptance and Mr Austen Chamberlain was appointed Chancellor of the Exchequer.

The appointment of Sir Auckland Geddes as he then was—he had been created K.C.B. in 1917—as Ambassador to the United States at a rather difficult post-war period was very unexpected and very critically received in many quarters owing to his lack of diplomatic training and experience. But he succeeded beyond expectation, though his mission in Washington came to an end in 1923 when he lost the sight of his left eye through an accident and subsequent unsuccessful operations for detachment of the retina.

On his recovery, after a period as Chairman of the Royal Commission on Food Prices, he became interested in commercial affairs, was Chairman of the Rio Tinto Company and the Rhokana Corporation with other directorships. The outbreak of war in 1939 found him again rendering public service as S.E. and later N.W. Regional Commissioner for Civil Defence until 1942. In that year he was seriously injured by a flying bomb and finally deprived of the sight of his remaining eye, operated on for cataract in 1941.

The remarkable career of Auckland Campbell Geddes has its setting in his family history as recorded by himself in the book already mentioned. The public careers and services of his two brothers and two sisters, scarcely less remarkable than his own, are summarized in a Review of Lord Geddes's book by the present writer (*Edinb. Univ. J.* 1952, 16, 125) as the immediate family background of their brother's special achievements. The theme of that book, as clearly indicated in the philosophical '*Testament at Seventy*' with which it concludes was the demonstration of 'a steady purpose in the converging influences of heredity, family tradition and

environmental circumstances culminating in the qualities and educational preparation of the five members of his generation for the work that they accomplished'.

Lord Geddes received many honours. The K.C.B. was followed by the G.C.M.G. in 1922 when he was Ambassador at Washington, and during that period he received the LL.D. from no fewer than ten Universities—McGill and Toronto in Canada and eight in the United States. His elevation to the peerage in the New Year's Honours of 1942 as Baron Geddes of Rolvenden was the fitting recognition of his very distinguished career.

The Anatomical Society, conscious of the unique distinction conferred on it by the public services of two former Professors of Anatomy, had elected Viscount Addison and Sir Auckland Geddes as Honorary Members together in 1926. J.C.B.

#### VISCOUNT ADDISON. K.G., P.C., M.D., B.S., F.R.C.S.

Christopher Addison, first Viscount Addison of Stallingborough, was born of Lincolnshire farming stock on 19 June 1869 and died at Radnage, High Wycombe, on 11 December 1951, full of years and honours. Though the major and more effective part of his career lay in politics, he began professional life as an anatomist, and devoted over twenty years to anatomical teaching.

From Trinity College, Harrogate, Addison entered Bart's in 1888, coming, in anatomy, under C. B. Lockwood, founder (and later President) of the Anatomical Society. He qualified M.R.C.S. (1891), M.B., B.S. London (1892) and M.D. London (1893), and took his F.R.C.S. (1895). Upon qualifying (at 22 years of age) he acted as Demonstrator of Anatomy at Bart's from 1891 to 1894 when he went as Professor of Anatomy to University College, Sheffield. There he reorganized and modernized the anatomical department. In 1901 he returned to London as full-time Lecturer in Anatomy to Charing Cross Hospital (whose Medical School he later served as Dean). In 1907 he returned to Bart's as first full-time Lecturer in Anatomy in its Medical College and remained in charge of the anatomical department until his abandonment of anatomy for politics in 1913. At the turn of the century, most metropolitan anatomical departments were officered by surgeons, established or apprentice: Addison therefore, like his friend and contemporary Arthur Keith, presented the unusual phenomenon of a well-qualified medical man intent upon whole-time devotion to academic anatomy. But whereas the Aberdonian was to remain faithful to his choice, the East Anglian had determined upon political life while still a young and relatively unknown professor in Sheffield. His return to London and his subsequent academic activities were therefore but means to this end: once anatomy had served his purpose it was abandoned for his prime interest. This doubtless explains why to Addison anatomy presented no problems save the merely utilitarian, but remained for him a static, purely factual subject: it explains also the pedestrian nature of his sole venture into research.

Addison examined in anatomy for the Universities of Cambridge and London and for the Royal College of Surgeons. As a teacher he was uninspiring, so that (it is said) when first a parliamentary candidate, his students, though of opposed political opinion, canvassed his cause in hope of ensuring his transference to Westminster.

In research, though devoid of anatomical flair or versatility and concerned mainly with matters of practical application, he was methodical, painstaking and accurate,

and his main investigation—his classic mapping of the topography of the abdominal viscera—reflects these traits. This pioneer work involved over 10,000 measurements on 40 cadavera and constituted the first reliable and precise guide to the relative disposition of the abdominal organs. Despite subsequent visceral studies by radiography and modern techniques, Addison's findings, in general, still hold for the body in the supine posture and his transpyloric plane has secured him eponymous immortality. The results of his visceral topographical studies were published in this *Journal* (1899–1901), and in the *Lancet*, *British Medical Journal* and book form (1901): they also formed the substance of Hunterian Lectures delivered in 1901.

Beyond editing the last (12th) edition of *Ellis's Demonstrations of Anatomy* (1905) and providing a preface to *Ellis's Anatomy* (1946), Addison contributed nothing further to anatomical literature.

Addison became a member of the Anatomical Society in 1895, served it as Honorary Secretary (1904–06) and in 1926 was elected an Honorary Member. In later life he received the honorary degrees of Sc.D. (Cambridge) and D.C.L. (Oxford) and was elected F.R.C.P.

In 1910 his political career opened with his election as Liberal M.P. for Hoxton. He became successively Parliamentary Secretary to the Board of Education (1914–15), Under-Secretary (1915–16) to Lloyd George as Minister of Munitions, Minister of Munitions (1916–17) under Lloyd George as Premier, and a Privy Councillor. In 1917 he became Minister in charge of Reconstruction and in 1918 the first Minister of Health. Differences of opinion with his leader led, in 1921, to his transference of political allegiance from the Liberal to the Labour party, and for some time he remained Minister without Portfolio. In 1929 he represented Swindon in the Commons in the Labour interest and from 1930 to 1931 was Minister of Agriculture under Ramsay MacDonald. Unseated in the 1931 general election he was later returned again for Swindon (1934–5) and in 1937 was raised to the peerage as Baron Addison of Stallingborough in the County of Lincoln, to become the effective leader of his party in the Lords. From 1945–7 he was Dominions Secretary and later Secretary for Commonwealth Relations. Created a Viscount in 1945, he received in 1946 his most prized honour, the Garter. Other high offices were his—Lord Privy Seal (1947), Paymaster-General (1948–9), and Lord President of the Council (1951). From 1948 onwards he was the official Labour leader in the Lords, attaining here perhaps his full political stature and by his personal qualities of candour, integrity and tolerance endearing himself to men of all parties.

Addison's abiding interest in social medicine, in agriculture and in medical education and research had its reflexion in his political activities. He supported Lloyd George in piloting the National Insurance Act through the Commons, and was responsible under Ramsay MacDonald for the first Agricultural Marketing Acts. A foundation member of the Medical Research Committee (1913–20) he was closely concerned with the establishment of its successor, the Medical Research Council, whose Chairman he became in 1948. It is due to Addison that this Council functions under the general direction of a special committee of the Privy Council, thereby enjoying an enhanced prestige and independence.

Addison's vigorous, clear, non-speculative mind was matched by a remarkable physical stamina which remained unimpaired to the last.

A.J.E.C.



## WILLIAM HENRY WOOD, B.Sc., M.D.

Derby Professor of Anatomy in the University of Liverpool, 1925-49

William Henry Wood died at his home in Aberdaron, Caernarvonshire, on 22 January, five years after retiring from the Derby Chair of Anatomy at Liverpool, which he had held for all but a quarter of a century. He was 66.

Wood received his early education at Lymm Grammar School, where he displayed an aptitude for the physical sciences and a precocious skill at both cricket and association football. His entry into the Victoria University of Manchester was, at least in part, in pursuit of opportunities to further his athletic interests, and choosing the Faculty of Science, he graduated B.Sc. in 1908, with Physiology as his principal subject. During his course he came under the inspiring leadership of the late Sir William Stirling, then at the height of his fame, and attracted his attention. A staff appointment in Physiology followed, and the three years for which he held it were among the happiest of his life, and happiest perhaps because they were the means of directing Wood's attention to Medicine, to which he decided to turn. He graduated M.B., Ch.B., in 1913. When war broke out Wood was among the earliest to enlist, and after training served as a casualty clearing-station officer with the R.A.M.C. in France during the early stages, being transferred later to Mesopotamia and afterwards to India.

Returning to Manchester when the war ended, a chance meeting with the late Sir Grafton Elliot Smith, then Professor of Anatomy at Manchester, led to an invitation to Wood to join the brilliant staff collected there, and it was as a member of this team of teachers and research workers that he developed his exceptional and powerful methods of teaching which were the basis of his success at Liverpool. Besides Stirling, for whom Wood had a deep affection, his idols were chiefly surgical: Platt, Milligan and the then young John Morley. For research as mere raw material for writing he cared nothing at all, and could not be persuaded to pen even a letter although exceptionally equipped to do so by ceaseless direct study and observation, research in the highest sense, from which rich well of understanding he drew constantly for the instruction of students. The quality of his work is perfectly reflected in the unpublished thesis on the anatomy of the small muscles of the hand awarded a gold medal in the M.D. degree examination of 1923. Now enshrined in orthopaedic practice, what he made clear about the hand and foot filtered through his teaching to the 'euthanasia' of scientific knowledge invented by Huxley as a solatium for those who establish the indisputable.

The care, supervision, guidance and instruction of students absorbed him. His deep ruling conviction was that the Profession of Medicine, which had developed Anatomy as a discipline separate from (however related to) generalized biological science, had a sort of legal *title* as the proper recipient of the Anatomist's services. The student himself was the most natural and immediate point of contact with the Profession. Wood's reward was certain, alike in the satisfaction with which he watched his students' successes and in the deep attachment which many generations



WILLIAM HENRY WOOD





of students retain for a beloved master. High moral courage, great strength of personal character and unswerving devotion to a single objective were among the grounds for an immense reputation among them. But also there was something not put into words but discerned by the few of his equals with whom he came into contact as co-examiners: an absolute reliance upon the truth that nothing can express itself in action of any sort, anywhere, but through the medium of some material embodiment: the philosophical fact that function is inherent in structure. Wood belonged to a generation of Anatomists reproached for exclusive addiction to the cadaver. The very remarkable thing about him, and possibly about them, was that for him, and perhaps for them, the cadaver was alive.

T.J.

## REVIEWS

*Problems in the Anatomy of the Pelvis, an Atlas.* By EDUARD UHLENHUTH. (Pp. xiv + 206; 66 plates and 16 text-figures;  $6\frac{1}{2} \times 9$  in.; £4.) Philadelphia: J. B. Lippincott Co. 1953.

This book is not and does not profess to be, a complete pelvic anatomy. Indeed the subject matter is very limited, the text is confined to 64 pages and is followed by 130 pages of plates. It deals with three aspects of pelvic anatomy.

Chapter 1 is devoted to the retrovesical and related spaces in the male with a description of their contents. Chapter 2 deals with the vesical trigone and urinary sphincters and chapter 3 with the layering of the levator ani.

The book is beautifully printed and produced, the text is clear and lucid and contains a large number of first-class illustrations of the author's own dissections, each with a comprehensive explanatory text. There is an adequate bibliography and the reader is also informed of the library where the references may be consulted.

A brief analysis of the third chapter will serve to illustrate the scope of the book. The pelvic floor is described as composed of two parts—the levator ani and coccygeus. The levator ani is split up into three parts—pubo-coccygeus, pubo-rectalis and ili-coccygeus. The pubo-coccygeus is then subdivided into five muscular bundles—pubo-prostatic (into the capsule of the prostate), pubo-bulbar (into the urethral bulb), pre-rectal (also into the capsule of the prostate), pubo-anal (distinct from pubo-rectal for it passes into the anal canal), retro-rectal (into the aponeurosis between the rectum and coccyx). The author emphasizes that the pre-rectal, pubo-anal and pubo-rectal muscles consist of diaphragmatic and anal parts, the latter forming a funnel-shaped envelope around the anal canal, thus the musculature closing the pelvic outlet is arranged in two planes, cranial and caudal and it is the former layer which forms the pelvic floor. We are, however, left wondering whether these divisions and subdivisions have a clinical significance or are merely of academic interest.

In chapter 2 the author tackles the vexed question of the sphincter mechanism of the bladder and supports his contentions by reference to his excellent dissections. It is interesting to note that he sides with Learmonth in stating that the sympathetic nerves are motor to the internal sphincter and inhibitory to the detrusor muscle and that the para-sympathetic nerves contract the detrusor muscle and relax the internal sphincter. He agrees with Learmonth that the internal sphincter possesses 'an intrinsic nerve mechanism' which gives it an 'inherent tonus' capable of closing the bladder when, for instance, the presacral nerve is severed, thus leaving the process of micturition intact.

The work as far as it goes is excellent but the author's choice of the problems of the pelvis is disappointing. Surely it is in the female pelvis that the problems abound. We want more evidence of the manner in which the female pelvic organs are supplied, of the formation and distribution of those ligaments of the pelvic floor about which there is so much controversy, we want to know more about sphincteric control in the female and especially of the precise relationship of the pubo-vaginalis portion of pubo-coccygeus to the urethra in order to help our surgical colleagues to construct a reliable operation for stress incontinence. We hope that Dr Uhlenhuth with his excellent dissecting technique will have an opportunity to turn his attention to some of these problems.

C. F. V. SMOUT

*Nature and Structure of Collagen.* Edited by J. T. RANDALL, F.R.S. (Pp. ix + 269; 142 illustrations;  $5\frac{1}{2} \times 8\frac{1}{2}$  in.; 42s.) London: Butterworths; New York: Academic Press. 1953.

This book contains some twenty-four contributions by various authors from the United Kingdom and Sweden on the morphology, molecular structure and biochemistry of collagen and related substances, presented for a discussion convened by the Colloid and Biophysics Committee of the Faraday Society. There could be no better example of the progress which can be made by physicists, chemists and biologists tackling a major problem in collaboration. A quite astonishing variety of techniques has been used, such as electron microscopy, X-ray, diffraction, radioactive isotopes, electrophoresis, chromatography, fibril reconstitution from solutions, infra-red absorption, tissue culture and routine protein analysis. Moreover, the techniques, have been combined in certain instances, so that the results from one approach can be checked by those from another. In fact, the onslaught is so comprehensive that this book gives an impression that some aspects of collagen, its molecular structure, formation, maintenance, ageing and diseases are in a few cases nearer solution than many of the problems of cytology at present investigated by the fashionable procedures of histochemistry.

Collagen has for some time been rather a dull subject amongst biologists. So stimulating is the recent work described in this book that few readers of this *Journal* can afford to neglect it. The 'colourful world of microscopy' is left far behind except in some of the introductory chapters. But for many of the investigations with which this book deals, visual evidence in monochrome is still indispensable. It seems a pity, especially for students approaching the conventional histology of collagen perhaps for the first time, that many of the photomicrographs should be of rather inferior quality. The same criticism indeed applies to quite a few of the electron micrographs, some of which appear to be out of focus. These defects are, however, quite trivial in comparison with the great interest of the contributions. At intervals, the papers are interleaved with informal discussions which give an impression of the personalities who attended the conference, even if they do not always clarify the issues raised.

K. C. RICHARDSON

*Atlas der Systematischen Anatomie des Menschen.* Band I. By GERHARD WOLF-HEIDEGGER. (Pp. iv + 218; Abb. 347;  $8 \times 10\frac{3}{4}$  in.; S. Fr. 32.) Basle: S. Karger Ltd. 1954.

The appearance of yet another atlas of Anatomy in these days of costly block making and printing may come as a surprise to many. It could be asked is yet another atlas necessary? The answer, of course, is that there is always a place for an atlas that is better than its predecessors.

The present volume is Band 1 of a series and deals with Osteology, Arthrology and Myology. The illustrations are well drawn and beautifully reproduced. It is unfortunate that a number of the figures have been reduced too much; e.g. fig. 76 of the hip bone could have been reproduced at almost twice its present size. Some of the drawings are not quite accurate, e.g. in fig. 325 the gemelli appear very much too massive and the fleshy belly of the obturator internus is carried too far laterally. These criticisms are made to help the author in the next edition.

The atlas is well reproduced and is a credit to the author, the artist and the publishers. One will have no hesitation in recommending it to medical students.

W. J. HAMILTON



*Guide to the Dissection of the Dog.* By MALCOLM E. MILLER. 3rd ed. (Pp. 369; 229 figures;  $11 \times 8\frac{1}{2}$  in.; 45s.) Ithaca, New York. 1952.

This book was written to aid veterinary students entering Cornell University 'to gain quickly and thoroughly a knowledge of basic mammalian structure and terminology'. The straightforward account given of the topographical anatomy of the dog should help in attaining this object.

The text is clearly written and abundantly illustrated by large line drawings though it is surprising not to find, for example, an adequate illustration of the orbital contents, nor one of the pterygoid region; the account of the abdominal contents is particularly good. An attempt is made to adhere to a systemic rather than a regional basis for dissection: many teachers would not agree that this is the best method of approach for a student. The sectional headings indicate the plan of the book (the number of pages is given after each): skeletal system (60); muscular system (61); thorax, neck and pectoral limb (52); abdomen, pelvis and pelvic limb (76); head (43); central nervous system (24); articulations (20). It is natural that a large amount of space should be devoted to the limbs, but a disproportion becomes noticeable when it is found that descriptions of arteries of the limbs occupy a total of about sixteen pages, whereas the arterial supply of the brain is dismissed in a single paragraph.

The format is unusual for a dissecting manual and its exceptionally large page size gives a length of line (nearly seven inches) which is uncomfortable for reading. Owing to the method of printing used the appearance is that of a bound typescript; this is accentuated by the inconsistent splitting of words (e.g. pter-ygopalatine, pterygopala-tine) which gives an impression of carelessness. Further, there is no differentiation of type and practical instructions are often not easily separated from the descriptive text.

There are many misprints (50 were noted) and some names are misspelt: Majendii. Descement for Descemet, and the author of Gray's *Anatomy* is referred to as Grey throughout. Such words as 'caudodorsolaterad' and 'proximodistal middles' seem almost to defeat their own object. The student may be confused by finding the eleventh cranial nerve variously called spinal accessory, accessory (spinal) or merely accessory. An isolated statement such as that on page 308 that 'Reinhard has recently made a thorough study of the venous drainage of the neuraxis of the dog' does not seem to have any place in a book of this sort, particularly as the only reference in the bibliography gives Reinhard, Karl; Personal Communication. A similar example appears on page 339.

A few of the figures, which are in general very satisfactory, call for comment. In fig. 59 the direction of the fibres of the superficial gluteal muscle and the relation of the middle gluteal muscle to the origins of tensor fasciae latae and sartorius are misrepresented. The labelling of the subscapular and suprascapular nerves should be reversed in fig. 104. In fig. 177 the positions of emergence of the spinal rootlets of XI and the ventral rootlets of the first cervical nerve are inaccurately shown in relation to those of nerves IX, X and XII.

The Index is inadequate. A random trial showed that the following are omitted: thoracic duct, basilar artery, rima glottidis, laryngeal sacculi, cavernous sinus, ventricular fold. Indeed neither 'fold' nor 'ventricle' is indexed; even the ventricles of the heart do not appear, 'heart' itself having but a single reference. Yet under the heading 'Bone(s)' there are 145 references among which may be mentioned: backbone; cremation of; depressions of; eminences of; epicardium (*sic*); hallux; nasion; pyriform aperture; vertebral head.

This is certainly a useful book and one to be welcomed for students beginning a study of mammalian anatomy, but the number of careless errors in this third edition, even though largely minor and topographical, points to the necessity for a very careful revision before the next edition. It is to be hoped that the aim mentioned by Prof. Miller in the Preface of writing 'a rather comprehensive text-book on the anatomy of the dog' will be achieved before long; the completion of such a work would be of great value. The present book is partly a by-product of this projected major work.

H. L. H. H. GREEN

*Schafer's Essentials of Histology.* Edited by H. M. CARLETON and R. H. D. SHORT. 16th edition. (Pp. xi+661; 659 figures, 3 plates. Price 35s.) London, New York, Toronto: Longmans Green and Co. 1954.

This book first published in 1885 has served its purpose well, but now in its 16th edition it is fast becoming a museum piece. In some ways it is a pity that so many students are still encouraged to use it at a time when the study of histology and cytology is receiving new impetus from the collaboration of chemists and physicists. The book survives, however, for several reasons. It has become a sort of symbol of histological teaching in departments of Physiology, so great is the prestige of its original author. Its price has remained modest, and it includes a certain amount of neuroanatomy, practical exercises, and details of technique which cannot be found altogether in other text-books of histology. Students required to learn somewhat less of the subject than medical students have to fall back on it for want of something more appropriate to their needs.

Despite its title, it contains a lot of inessential detail for a book of such scope; detail which is unrelieved by emphasis in the text as to its relative importance. Then there is the bewildering confusion of styles amongst the illustrations, unhappily increased in this present edition by the impressionistic pen sketches of the junior editor. Is it any wonder that the average student so often feels the need for an effort of imagination when attempting to equate what he sees under the microscope with his textbook illustrations? In many details this present edition fails to bring the subject up-to-date. Perhaps this is not entirely the fault of the editors. Indeed, one suspects that the publishers have given them so little latitude that no room could be found even for an electronmicrograph or two illustrating the essential characteristics of collagen fibres. As in previous editions we note, for instance, that the blood circulates intelligibly in the cortex of the kidney (figure 469), but its course in the medulla is still somewhat ambiguous. There are lots of inconsistencies of this kind and one wonders how many teachers of histology have had to spend unnecessary effort in explaining to students who use this book the old-fashioned confusion between endothelium and epithelium, the misinterpretation of cortex and medulla in the mouse adrenal according to Cramer, the nomenclature applied to keratin precursors in the epidermis, the supposed existence of intracellular spaces in liver cells communicating with the sinusoids, and several somewhat dubious instances of direct innervation concerning blood capillaries (figure 252), the adeno-hypophysis and the islets of Langerhans. There must be few teachers of histology in Great Britain and the Commonwealth who have not had an affection for this book in the past. To some it must be painful to realize that short of drastic revision in the light of recent progress, the life of this book is fast ebbing under the tinkering treatment it has received in post-war editions.

K. C. RICHARDSON

## BOOKS RECEIVED

*The Adaptive Chin.* By E. LLOYD DuBRUL and HARRY SICHER. (Pp. ix+97; 47 figures; 25s.) Oxford: Blackwell Scientific Publications. 1954.

*Die objektive Stereoskopie an Röntgenbildern.* By A. HASSELWANDER. (Pp. viii+187; 125 figures; DM. 27.) Stuttgart: Georg Thieme Verlag. 1954.

*Proceedings of the Society for the Study of Fertility.* Number v, Liverpool Conference, 1953. Cambridge: W. Heffer and Sons Ltd.

*The Physiology of Man.* By L. L. LANGLEY and E. CHERASKIN. (Pp. xii+608; 180 figures.) London: McGraw-Hill Book Company Inc. 1954.





# THE ZONA INTERMEDIA OF THE ADRENAL CORTEX. A CORRELATION OF POSSIBLE FUNCTIONAL SIGNIFI- CANCE WITH DEVELOPMENT, MORPHOLOGY AND HISTOCHEMISTRY

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## INTRODUCTION

In the course of their work on the effects of castration upon the adrenal cortex of the rat, Hall & Korenchevsky (1937) described a 'demarcation zone', 1-4 cells wide, between the zonae glomerulosa and fasciculata of the normal animal. The cells of this zone, they claimed, contained little or no lipid in contrast to those either side of it; in castrated rats, however, this fat-free zone became lipid positive.

Earlier, Reiss, Bálint, Oestreicher & Aronson (1936-7), in presenting a method of assay for ACTH in the rat, described a sudanophobe zone as the outer part of the z. fasciculata; this fat-free zone, broader in hypophysectomized animals, disappeared when these were treated with ACTH. This work was later to be confirmed by that of Simpson, Evans & Li (1943).

Tobin & Whitehead (1941-2) described a sudanophobe zone immediately internal to an outer fat-laden zone in the rat's adrenal cortex. In describing the cells of this sudanophobe zone in the rat, Greep & Deane (1947) used the term 'transitional zone', while Mitchell (1948) preferred to call it the zone of 'compression' because the number of cells per unit area was increased within it in comparison with the rest of the gland. Other authors, while recognizing the zone, have spoken of it as the outermost part of the z. fasciculata (Dalton, Mitchell, Jones & Peters, 1943-4), or included it in with the z. glomerulosa (Harrison & Cain, 1947). Nicander (1952) referred to it as the 'intermediate zone' since the component cells appeared to him intermediate in type between those of the zonae glomerulosa and fasciculata in a large number of the domestic animals. Yoffey & Baxter (1947), examining rat adrenals for birefringent material, reported a region of optical inactivity between the zonae glomerulosa and fasciculata. Furthermore, Harrison & Cain (1947), in the rat adrenal, found cholesterol in the zonae glomerulosa and fasciculata, but not in the sudanophobe zone.

Cain & Harrison (1950), in the rat, described the cytological features of the sudanophobe zone as similar but weaker than those of the z. glomerulosa, and commented that while there was an obvious reduction in cytoplasm in the former zone, the nuclei of both zones were the same size. They postulated that a cell movement from the sudanophobe zone was predominantly outwards to the z. glomerulosa through intermediate stages, while sharp cytological differences between the sudanophobe cells and those of the z. fasciculata suggested little inward passage from the sudanophobe zone. This idea of the sudanophobe zone in the rat being a generative region

is contradicted by the work of Mitchell (1948), who described a mitotically inert zone of the rat cortex between the z. glomerulosa and z. fasciculata. Cater & Stack-Dunne (1953) observed mitoses throughout the rat cortex, but with the lowest incidence in the sudanophobe region. Hoerr (1931), studying the reactions to injury by chloroform narcosis in the guinea-pig, noted an increased mitotic count principally in the z. fasciculata, with only a few mitoses in the z. glomerulosa; but it must be added that Hoerr did not recognize a sudanophobe or intermediate zone. Dalton *et al.* (1943-4) regarded the sudanophobe zone as a region of lipid release; according to them fat-laden cells of the z. glomerulosa, migrating centripetally, became depleted of lipid in this sudanophobe zone. This belief was largely based on the conception of a capsular or subcapsular origin for cortical cells which then migrated inwards to degenerate in the z. reticularis (Zwemer, 1933-4; Zwemer, Wotton & Norkus, 1938; Bachmann, 1939; Wotton & Zwemer, 1943).

The incidence of the sudanophobe or intermediate zone is the subject of some considerable controversy. Thus Feldman (1951) described it as variable and inconstant in the rat. Harrison & Cain (1947), also in the rat, found the zone present in immature animals of both sexes and usually present in mature males, but absent in adult females, and male castrates. Cain & Harrison (1950) later reported a sudanophobe zone in 123 out of a series of 154 rat adrenals (from immature animals) examined by the acid haematein method. Tobin & Whitehead (1941-2), while claiming the presence of a sudanophobe zone in the rat adrenal, reported its absence in the glands of the mouse, guinea-pig and rabbit. Nicander (1952) described an intermediate zone in a wide variety of the domestic animals, but claimed it to be sudanophobe only in the horse, dog and rat; according to him it was weakly sudanophile in the mouse, guinea-pig and in cattle, and definitely sudanophile in the cat and rabbit. Knouff & Hartman (1951), although they claim a zoning into glomerulosa, fasciculata and reticularis of the cortex in the brown pelican, do not mention a z. intermedia.

Mitchell (1948), describing the development of the rat adrenal, observed a 'zone of compression' just internal to the glomerulosa during the first postnatal week; by the second week the cells of this zone were basophilic and could be clearly seen interposed between glomerulosa and fasciculata.

Cain & Harrison (1950), in the rat, claimed that the sudanophobe zone was less vascular than the z. glomerulosa. Earlier, Popják (1944) had observed a hyperaemic zone between z. glomerulosa and z. fasciculata in rat adrenals, 24 hr. after leg crush injuries. However, Bennett & Kilham (1940), observing a narrowness of the capillaries in the z. fasciculata in the cat, believed this to be due to compression by the parenchymal cells of the zone which were turgid with lipid. Lever (1954), after intravital ink injections into the left ventricle in rats, demonstrated a zone of comparatively poor capillary filling corresponding to the z. intermedia plus a variable width of the outer fasciculata. He contends that the sharp outer edge of this zone corresponds to the external limit of the z. intermedia, while the inner edge, more irregular, probably corresponds to a succession of points where the intracellular lipids of the z. fasciculata are reduced in amount.

It is generally agreed that the sudanophobe zone in the rat becomes greatly widened following hypophysectomy. Lever (1954) demonstrated a linear increase with time in the extent of the intermediate or sudanophobe zone after hypophysectomy.

Following ACTH treatment in hypophysectomized animals, Reiss *et al.* (1936-7) and Simpson *et al.* (1943) described a re-establishment of the normal orderly arrangement of zones in the adrenal, a fact employed by them in their methods of assay of ACTH.

In this paper the term *z. intermedia* is used to indicate the anatomical location of a zone which may be present between the *zonae glomerulosa* and *fasciculata*. As will be shown later, it can be both absent or very clearly defined in the rat adrenal under different physiological conditions.

## MATERIALS AND METHODS

### A. Comparative morphology

In an attempt to find a homologous arrangement to the intermediate and sudanophobe zones described in some mammalian adrenals, the non-mammalian adrenal was studied in two of each of the following animals: the domestic fowl, missel-thrush, grass-snake, adder, crocodile, green lizard, salamander (*Salamandra maculosa*), common newt and frog (with the exception of the fowl, all were male animals). One adrenal was fixed in Susa and stained with haematoxylin and eosin, and the other was fixed in 4% neutral formaldehyde, freeze-cut, and stained with Sudan black for lipids. In the salamander and newt the diffuse arrangement of the glands along the ventrimedial borders of the kidneys makes separation difficult, and left and right adrenals were examined together.

In a short series of some readily available mammals (Tables 1 and 2), left-sided adrenals were stained with Sudan black for lipid and right-sided adrenals were stained with haematoxylin and eosin.

### B. Development and age changes in the rat's adrenal

Forty-eight white Wistar rats (from the same stock as used for the histochemistry and experiments below) were examined. These included eight pregnant rats and the foetuses, two rats at birth, and at 1, 2, 3 and 4 days after birth; one rat at 5, 6, 7, 8, 10 and 14 days; a male and female rat at 3, 4, 5, 6, 7 and 8 weeks; male rats at 3, 4, 5, 6, 9 and 12 months. One adrenal from each rat was fixed in Baker's calcium-formol saline, and frozen sections cut for lipid studies. The other adrenal was fixed in Susa, paraffin embedded, and serial sections were stained with haematoxylin and eosin. For the mitotic counts, representative mid-sections were projected at a magnification  $\times 100$  and drawn on squared paper. The sections were examined with a 2 mm. objective for mitotic figures, the positions of which were plotted on to the projection. The stage of each mitotic figure was also noted. Three adjacent sections were read, and the results of all three were recorded on the one projection to avoid errors of sampling.

### C. Histochemistry of rat's adrenal

This was studied on rats treated as follows: normal (21), castrated 14 days (4), hypophysectomized (105), hypophysectomized and castrated (8). The hypophysectomized rats were studied at various times after operation: after 1 day (20), 2 days (18), 14 days (18), 19 days (1), 21 days (24), 24 days (5), 28 days (5), 35 days (1),



42 days (1), 49 days (1), 56 days (1), 67 days (4), 117 days (3). Half the hypophysectomized and castrated rats were examined after 14 days and half after 21 days. The two adrenals from each rat were separately treated to increase the range of histochemical study, which included the techniques listed below:

(1) *Lipids*. One hundred and thirty-three adrenals, fixed in Baker's calcium-formol saline, were cut with a freezing microtome; one section was stained with Scarlach R, one with Sudan black, and one examined unstained with the polarizing microscope.

(2) *Schultz's method*. Thick (25  $\mu$ ) frozen sections of calcium-formol-saline-fixed material (103 adrenals) were examined by a modified Schultz's method for cholesterol.

(3) *Baker's acid-haematein method* (1946) was used on ten adrenals to demonstrate phospholipids.

(4) *Nile blue* (Cain, 1950). Ten adrenals were examined by this technique after post-chroming.

(5) *Plasmal reaction*. Ten adrenals were rapidly frozen and kept at  $-20^{\circ}$  C. until required. Unfixed frozen sections were obtained by using a knife cooled with CO<sub>2</sub> snow. They were dropped into the plasmal reagent (equal parts of (a) Schiff's solution half diluted with bisulphate water and (b) saturated 7% aqueous mercuric chloride solution). After washing with sulphurous acid and distilled water, they were mounted in glycerine jelly. Control sections were stained in Schiff's solution.

(6) *Nucleic acids*. Ten adrenals fixed in Susa were examined by the Feulgen method for desoxyribonucleic acid (Feulgen & Rossenbeck, 1924) and by pyronin-methyl green mixtures for ribose nucleic acid. Unfortunately, ribonuclease was not available.

(7) *Periodic acid-Schiff* (P.A.S.) *method* (McManus, 1948). Twelve adrenals fixed in ice-cold 80% ethanol were examined by this method. Freeze-dried vacuum embedded material was also examined.

(8) *Ascorbic acid*. Thirty adrenals were treated by the method of Barnet & Bourne (1941-2). Six rats hypophysectomized 24 hr. previously had the left adrenal removed and fixed in silver-acetic acid. The rats were then injected intravenously with a high, medium, or low dose of ACTH (Armour batch 84-85 H., 1.6, 0.1 or 0.006  $\mu$ g./100 g. rat respectively). After 1 hr. the right adrenal was removed and placed immediately in the same fixative. In another experiment, nine hypophysectomized rats were injected with ACTH and 1 hr. later both adrenals were removed so that both the ascorbic acid and lipid distribution could be compared with control adrenals (five hypophysectomized, and five normal rats).

(9) *Alkaline phosphatase*. Ten adrenals fixed in ice-cold 80% ethanol, and five adrenals freeze-dried and vacuum embedded, were examined by the Gomori technique (Danielli, 1946).

(10) *Acid phosphatase*. Six adrenals fixed in ice-cold absolute acetone and five freeze-dried adrenals were examined by the Gomori (1941) technique.

(11) *Esterase*. Six adrenals fixed in ice-cold absolute acetone, five freeze-dried adrenals, and two unfixed adrenals cut by the cold-knife method, were examined by the technique of Nachlas & Seligman (1948-9).

*D. Rat adrenal in various physiological and experimental conditions*

Rats used for studying (i) the effect on the adrenal of hypophysectomy, and (ii) the effect of ACTH on the adrenal ascorbic acid distribution, have been detailed above. The lipid distribution 1½, 4, and 8 hr. after intravenous injection of ACTH (at three dose levels as above) was studied on eighteen rats hypophysectomized 2 days previously. In each case the left adrenal was removed as a control before the injection of ACTH. Twenty rats were used to study the effect of castration on the adrenal. In addition to histochemical examination of these adrenals as outlined above, routine histological examination was made of seventy-one adrenals, fixed in Susa and stained with haematoxylin and eosin.

## RESULTS

*A. Comparative morphology**(1) Observations on some non-mammalian adrenals*

It is not claimed that a full examination of the adrenal cortical homologues was made, but such material as was available provided useful information.

*The frog adrenal.* The cortical elements are arranged in short irregular cords extending into the gland from the deep surface of the capsule (Pl. 1, fig. 1). The cells at the capsular end of the cords have an eosinophilic, homogeneous cytoplasm and a round to oval reticular nucleus with a nucleolus. The cells next encountered appear crowded and may constitute a zone of cell-compression. They have spindle-shaped nuclei, elongated at right angles to the axis of the cord, and their cytoplasm is scanty and eosinophilic. Immediately internal to these cells, the cord consists of larger cells with round reticular nuclei and foamy cytoplasm reminiscent of the spongiocyte cells in the mammalian outer z. fasciculata. These appearances in the frog are strikingly parallel to those seen in the outer cortex of certain mammals, e.g. the rat (Pl. 1, fig. 3). The presence of what appears to be a z. intermedia in a non-mammalian adrenal is of great interest. Lipoid studies on the frog adrenal (Pl. 1, fig. 2) are also suggestive of a zoning in the cortical cords. While the spongiocytes in the middle of the cortical cords have a high lipid content, the cells at both the outer and inner ends of the cords have a relatively low lipid content. In examination of lipoid preparations it should be remembered that the cells of the medullary homologues lie between the groups of cortical cells, contain no lipids, and may appear as unstained interruptions in a region of stained tissue.

*Urodele adrenal.* In the rather diffuse adrenals of the Urodeles no zoning could be observed in the loose masses of cortical cells, in either cytological or lipoid preparations.

*Reptilian and Avian adrenals.* In the reptilian gland there is no cytological zoning within the branching columns of cortical cells. While the adrenal of the domestic fowl has a cortical arrangement very similar to the reptilian gland, it must be added that pyknotic nuclei are more common in the central than in the peripheral portions of the cortical-cell cords. This is interesting in view of the work of Knouff & Hartmann (1951) who described zonae, glomerulosa, fasciculata, and reticularis in the adrenal of the brown pelican.

(2) *Observations on the zona intermedia of some mammalian adrenals*

(a) *Cytological comparisons.* In the following comparative study, a *z. intermedia* is judged to be present if there is interposed between the *zonae glomerulosa* and *fasciculata* a tissue layer, one or more cells thick, which is clearly unlike either of these zones. Differences of cell size, nuclear shape, and cytoplasmic staining and appearance, were collectively or individually, the distinctive criteria.

In view of the theory of a capsular origin for cortical cells and their centripetal migration to the *z. reticularis* (Zwemer, 1936; Zwemer *et al.* 1938; Wotton & Zwemer, 1943) the cellular arrangement of the capsule and the *z. glomerulosa* was studied. In some instances (cow, sheep, ox) there is no very sharp distinction between *glomerulosa* cells and those in the deeper layers of the capsule, while in others (horse, cat, mouse, rat and rabbit), the distinction is obvious.

The cell arrangement in the *z. glomerulosa* is very variable in different animals; thus in the horse, sheep and mouse, single columns or double columns with arched ends, are observed (Pl. 1, fig. 4), while in the cat and ox the cells are arranged in small clusters or rosettes (Pl. 1, fig. 5; Pl. 2, fig. 11). In other animals (pig, cow, guinea-pig) no one cell arrangement predominates and this can be described as diffuse.

All mammalian material was examined with particular reference to the intermediate region between the *z. glomerulosa* and the *z. fasciculata*, and the results of this comparative study of the *z. intermedia* are summarized in Table 1.

The *z. intermedia* is most commonly displayed as a region where the number of cells per unit area is greater than in other zones of the cortex (Pl. 1, figs. 5-7). This is probably not an invariable feature of the zone as is indicated by the example of the cow in Table 1. Again in some adrenals, notably those of the rat, sheep and ox, the cytoplasm of the *z. intermedia* is often markedly basophilic (Pl. 1, fig. 8), while in other animals (cat, guinea-pig, horse) this is not so.

The *z. intermedia* appears to constitute a region of cellular re-arrangement and change from the *glomerulosa* to the *fasciculate* patterns. In many animals the nuclei in the *zonae glomerulosa* and *intermedia* are similar, even though there may be cytoplasmic reduction in the latter zone. However, in the *z. intermedia* of the horse, rabbit and sometimes of the pregnant rat, a flattening is observed of both cell and nucleus, at right angles to the radius of the gland (Pl. 1, fig. 3). Flattened cells without nuclei in the plane of the section are also seen, suggesting a real spread of the cell cytoplasm (Pl. 1, fig. 9). These observations may indicate cell compression, as suggested by Mitchell (1948) in the rat, though he did not report nuclear flattening.

From a study of Table 1 the following observations can be made: (a) In the rat, cat, dog, sheep, rabbit and horse where a definite *z. intermedia* is clearly present, the cell arrangement of the *z. glomerulosa*, in addition to being typical for each animal, is regular (Pl. 1, figs. 4-7). (b) Where the arrangement of the *z. glomerulosa* is diffuse, or irregular, then the *z. intermedia* is either, not detectable as in the pig, or not well demonstrated as in the cow and guinea-pig (Pl. 1, fig. 10). The ox and mouse adrenals do not fit into these generalizations. The ox adrenal, though it has a fairly obvious *z. intermedia* does not show a very regular arrangement in the *z. glomerulosa* (Pl. 2, fig. 11). In contrast, the mouse adrenal, though possessing



a regular form in the z. glomerulosa, does not have an obvious z. intermedia (Pl. 2, fig. 12). However, the cell count in the mouse adrenal shows an increase per unit area between  $64\mu$  and  $95\mu$  under the capsule, and the inner edge of the z. glomerulosa lies at  $70\mu$  below the capsule (as measured with a micrometer eyepiece).

Table 1. *A comparison of the zona intermedia in some common mammals*

Column 2: the presence of a z. intermedia is indicated by a +. When the zone is particularly well developed this is shown by ++. Column 3: each haematoxylin and eosin section was projected on to  $\frac{3}{4}$  in. squared paper at a magnification of  $\times 576$  and nuclear counts were made for a variable number of squares from the capsule into the z. fasciculata. An increase in the cell count over a limited area in the outer cortex is indicated by +. (In the horse adrenal a variable amount of connective tissue between the columns of outer cortical cells rendered a cell count valueless.) Column 4: indicates the distance in  $\mu$  beneath the capsule of the outer and inner margins of the square in which the maximum cell count was observed. Column 5: indicates the distance in  $\mu$  from the capsule to the centre of the z. intermedia (mean of eight measurements on camera lucida tracings). When these values lie within the range indicated in column 4 there is a greater significance in a positive rise in the cell count. Column 7: + indicates a detectable amount of lipids; ++ indicates larger quantities of lipids.

Animals and number used (1)	Detected incidence of z. intermedia (2)	Cell count result (3)	Range in $\mu$ below capsule of maximum cell count (4)	Centre of z. intermedia below capsule in $\mu$ (5)	Arrangement of cells in z. glomerulosa (6)	Z. intermedia lipids present or absent (7)
Dog 1	++	+ 've	190 230	200	Regular	—
Horse 2	++	Unreadable		220	Regular	—
Rat 12	++	+ 've	64 95	80	Regular	—
Rabbit 4	++	+ 've	130 160	120	Regular	++
Sheep 2	++	+ 've	190 230	200	Regular	+
Ox 1	+	+ 've	130 160	150	Equivocal	+
Cat 3	+	+ 've	64 95	80	Regular	++
Guinea-pig 2	+	+ 've	34 64	40	Diffuse	+
Cow 2	+	— 've	.	160	Diffuse	+
Mouse 2	—	+ 've	64 95	.	Regular	+
Pig 1	—	— 've	.	.	Diffuse	.

(b) *Comparison of lipid distribution in outer cortex.* Table 2 shows the lipid distribution, as indicated by Sudan black, in the same range of domestic animals. It is noteworthy that in the rabbit and cat, where the z. intermedia is strongly sudanophilic, that the glomerulosa lipids are sparse (Pl. 2, fig. 13); while in the rat, dog and horse in which the z. intermedia is sudanophobe, the glomerulosa lipids are usually present in large quantity (Pl. 2, figs. 14, 21 and 22). Furthermore, in the cow, ox and sheep small quantities of lipid are equally present in the zonae glomerulosa and intermedia, while in the mouse and guinea-pig there is an upward lipid gradient from the z. glomerulosa through the z. intermedia to the outer z. fasciculata. In the pig the z. glomerulosa appeared completely devoid of lipid in contrast to the heavy content of the z. fasciculata. It will be remembered that the z. intermedia was not detected in the pig adrenal.

Table 2. *A comparison of the lipoid distribution in the outer adrenal cortex of some common mammals*

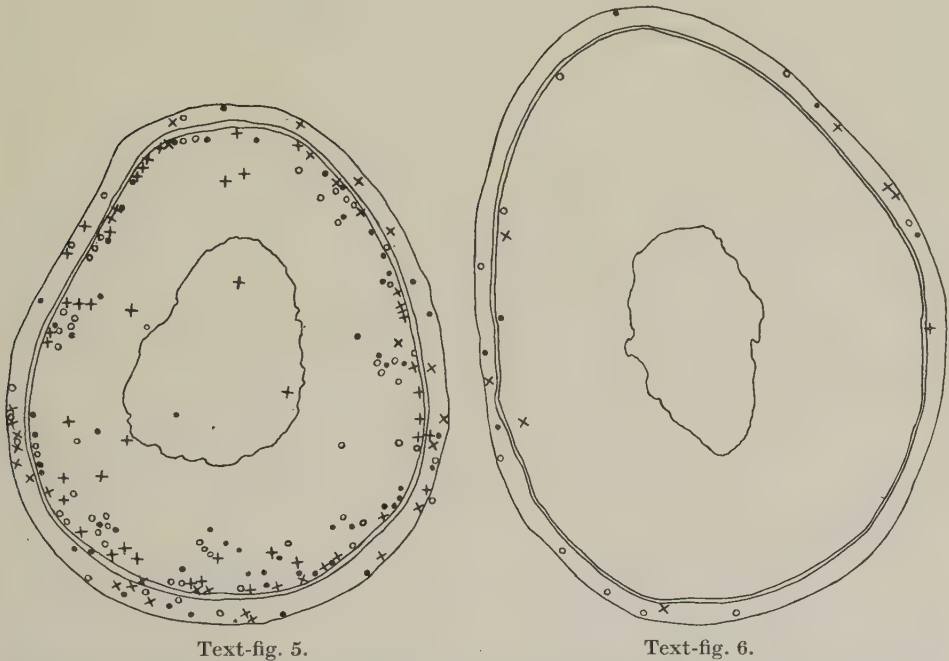
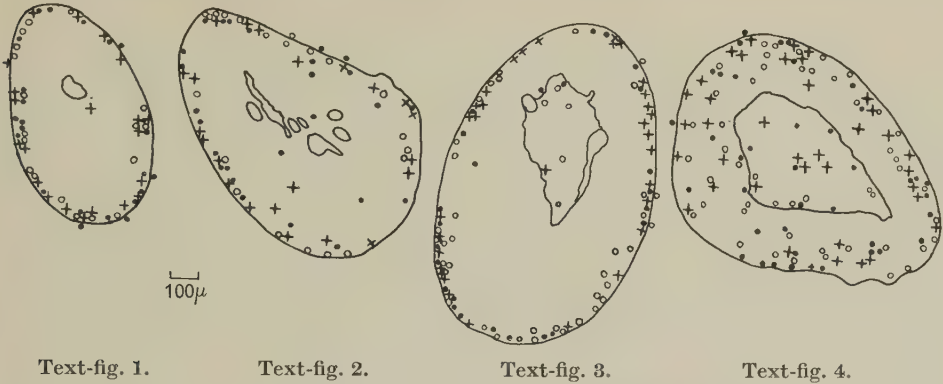
The lipids were visualized by staining with Sudan black. A single + indicates a definite but light lipoid distribution, while ++ indicates a heavy lipoid distribution. Absence of lipid is denoted by -. *Sparse* is a term used to denote an almost fat-free region which however contains a very few lipid droplets.

Animal	Z. glomerulosa lipids	Z. intermedia lipids	Outer z. fasciculata lipids
Dog	++	-	++
Horse	++	-	++
Rat	++	-	++
Cat	Sparse	++	++
Rabbit	Sparse	++	++
Cow	+	+	++
Ox	+	+	++
Sheep	+	+	++
Guinea-pig	Sparse	+	++
Mouse	Sparse	+	++
Pig	-		++

### *B. Development and age changes in the rat's adrenal*

The earliest stages of development studied were the adrenals of 1 and 1.8 g. embryos (approximately 16th and 18th days of pregnancy respectively). In these, mitotic figures are present in the gland capsule. Beneath the capsule there is a layer 1 to 3 cells thick containing numerous mitotic figures (Text-fig. 1). The cells of this layer have a small amount of basophilic cytoplasm containing a little lipid. The remainder of the adrenal cortex consists of a zone of larger lipid-laden cells (undifferentiated zona fasciculata) enclosing a central mass which is the foetal cortex. The cells of this foetal cortex are large, eosinophilic and fat-free (Pl. 2, figs. 15, 16). Mitotic figures are present in the outer part of the z. fasciculata in 1 and 1.8 g. embryos. In the 4.5 g. embryo (approximately 20th day of pregnancy) the outer zone of dark-staining cells beneath the capsule is more obvious, but the mitotic figures are more widely scattered and include some in the deeper layers of the cortex (Text-fig. 2). The foetal cortex is no longer seen, but the medulla is forming as a loose central mass by the aggregation of islands of chromaffin cells. At birth (5-6.5 g. rats) there is little lipid present in the outermost zone of the cortex, but by the 3rd day (9 g.) a lipid-containing z. glomerulosa and a lipid-free z. intermedia are recognizable (Pl. 2, figs. 17, 18; Text-fig. 3). The lipid-laden but undifferentiated z. fasciculata internal to the z. intermedia exhibits a fasciculate pattern at 7 days. At about the 10th day most of the lipid in the z. fasciculata is in its outer layers and there is a lipid-free zone bordering on the medulla (Pl. 2, fig. 19; Text-fig. 4). The z. reticularis is apparent by the 21st day and about this stage the z. intermedia appears narrower. The typical pattern of zones as seen in the adult is present by about the 35th day. Between the 28th and the 35th day the mitotic activity in the adrenal declines rapidly in this series (compare Text-figs. 5 and 6). There is no indication that the z. intermedia is the mitotic zone of the cortex; on the contrary, mitotic figures can be found in all zones and in the capsule. In Text-fig. 5 there is one mitosis in the capsule, forty-seven in z. glomerulosa, three in z. intermedia, ninety in the outer part of z. fasciculata, forty-nine in the inner part of z. fasciculata and four in the z. reticularis.

*Age changes in the rat's adrenal.* From the 2nd to the 4th month the z. intermedia becomes less distinct or may be altogether absent (Pl. 3, fig. 29). In the lipid preparations at this age, the zone is usually sudanophile (Pl. 2, fig. 20). However, by the 6th month the z. intermedia is again usually sudanophobe, and in addition can



Text-figs. 1-6. Mitotic activity in rat adrenal cortex at different ages. Each fig. represents the readings of three consecutive sections; key: 1st section, +; 2nd section, •; 3rd section, ○. Scale: 1 cm. = 280μ. Text-fig. 1, 1.8 g. foetus; Text-fig. 2, 4.5 g. foetus; Text-fig. 3, 9 g. male, 3 days old; Text-fig. 4, 14 g. male, 10 days old; Text-fig. 5, 54 g. male, 28 days old; Text-fig. 6, 57 g. male, 35 days old.

once more be identified in haematoxylin and eosin preparations by the small size of its cells. The z. intermedia becomes increasingly well defined with age (Pl. 2, figs. 21, 22) so that in the senile rat adrenal it is a region of marked cell flattening (Pl. 3, fig. 30). The above description applies particularly to the male rat. Our



observations (though limited) on the female rat indicate the presence of a z. intermedia in haematoxylin and eosin preparations from all age groups. Corresponding lipid studies indicate that the zone contains a few fine lipid droplets in the newly matured rat (2–6 months), but is lipid free in younger and older female rats.

### C. Histochemistry of the adult rat's adrenal

*Lipids.* The z. intermedia is usually not stained (see above) by the Sudan stains Sharlach R and Sudan black, but when it is sudanophile the polarizing microscope shows no anisotropic lipid and the Schultz method for cholesterol is negative (Pl. 3, fig. 23). It must, however, be noted that neither of these tests is sufficiently sensitive to detect small quantities of cholesterol. The Baker method for phospholipids shows heavy staining in the z. fasciculata and some in the z. glomerulosa, but the z. intermedia is unstained. In the adrenal of the hypophysectomized rat the much wider z. intermedia or sudanophobe zone, forms a conspicuous unstained band in sections stained by the Schultz method (Pl. 3, fig. 24), and the Baker method (Pl. 3, fig. 25).

*The plasmal reaction.* Fresh unfixed frozen sections of adrenals, placed in a mixture of Schiff's reagent and mercuric chloride, gave a strongly positive plasmal reaction in the z. glomerulosa and z. intermedia (control sections placed in Schiff's solution were unstained). Similar results were obtained with the adrenals from rats hypophysectomized 14 days previously, in which these zones are relatively much wider. This finding must be accepted with caution. It is not easy to obtain satisfactory frozen sections of unfixed tissues cut by the cold-knife technique. It is possible that diffusion and smearing artefacts can occur in the unfixed tissues. On the other hand, it is equally possible that soluble materials may be leached out of the z. intermedia by aqueous fixatives or inactivated by formaldehyde. The phenylhydrazine reaction, introduced by Bennett (1940), for ketosteroids, probably stains the plasmals of the adrenal cortex (Gomori, 1942, 1952). Rogers & Williams (1947) calculated that the quantity of ketosteroids in the adrenal cortex is too small to be demonstrated histochemically. Pearse (1953), reviewing the subject, suggests that formalin fixation or auto-oxidation in air, liberates aldehydes from the unsaturated fats in the adrenal cortex, and that these aldehydes give both the plasmal and phenylhydrazine reactions. Because of these doubts concerning the reactions for ketosteroids none are described here.

*Fixation artefacts.* The possibility was considered that the small size of the cells in the z. intermedia and their sudanophobe nature might be due to the loss of soluble substances from their cytoplasm during fixation. In freeze-dried vacuum-embedded sections of the normal and hypophysectomized rats' adrenal, the cells of the z. intermedia are still small relative to those of the adjacent zones. Unfixed frozen sections, stained with Sudan black or examined with the polarizing microscope, show no definite evidence of lipids in the z. intermedia. Each of these methods is open to criticism, and all that can be said is that no positive evidence has been obtained to date to indicate that the z. intermedia is a fixation artefact. An intra-vital perfusion experiment was therefore performed, 5–8 ml. of 0.5% methylene blue in citrated normal saline being perfused into the femoral veins of rats. Death, due to circulatory failure, occurred in 10–20 min. and the adrenals were immediately

freeze-cut (without fixation) and photographed. Pl. 3, fig. 26, clearly indicates that the methylene blue has stained the z. glomerulosa and z. fasciculata but has not stained the z. intermedia. The experiment was repeated in the rabbit, in which the z. intermedia is found to be sudanophil; the zone was heavily stained by the methylene blue.

*Pyronin-methyl green stain.* The cells of the z. glomerulosa and z. intermedia stain with pyronin, and this confirms the basophilia of these cells seen after staining with haematoxylin and eosin. Study of the adrenals of rats hypophysectomized 2 weeks previously, confirms that the cells which stain strongly with pyronin are confined to the z. glomerulosa and intermedia (Pl. 1, fig. 8).

*The periodic acid-Schiff (P.A.S. method).* No more than traces of P.A.S. positive material are seen in adrenals of normal or hypophysectomized rats either in alcohol fixed or freeze-dried material.

*Ascorbic acid.* In both control, and ACTH-treated adrenals, the outer cells of the z. glomerulosa are practically free from the silver granules which indicate the presence of ascorbic acid. Intracellular silver granules are present in the inner part of the z. glomerulosa, but they are larger and more numerous in the z. intermedia (Pl. 3, fig. 27). In the cells of the z. fasciculata the silver granules are very large in the control adrenals, but after medium and large doses of ACTH become reduced in number and smaller in size. In the outer part of the z. fasciculata the capillaries show numerous fine silver granules lining their walls and in their lumina, presumably indicating that the ascorbic acid is leaving the cells (Pl. 3, fig. 28). This appearance occurs in some of the control adrenals in the z. reticularis and innermost portions of the z. fasciculata, but is only seen in the outer fasciculata after treatment with ACTH. The silver granules in the z. intermedia do not appear to be altered by treatment with ACTH in this experiment. Our findings are similar to, but not identical with, those of Deane & Morse (1948), and Greep & Deane (1949).

*Enzymes.* These histochemical reactions for acid phosphatase, alkaline phosphatase, and esterase, did not outline the z. intermedia and distinguish it from the adjacent zones. The staining for these enzymes is, however, rather stronger in the zonae glomerulosa and intermedia than in the z. fasciculata, but with these techniques only well-marked differences probably have any significance.

#### D. Rat adrenal in various physiological and experimental conditions

The zona intermedia in the male rat is more obvious than in the female rat of corresponding age, this observation concurs with that of Greep & Jones (1950). Castration of the male rat results in an adrenal simulating the female type. In both female and castrated male adrenals the z. intermedia can still be distinguished in haematoxylin and eosin preparations. Marked flattening of the cells and nuclei in the z. intermedia is frequently seen in adrenals from rats in late pregnancy (Pl. 1, figs. 3, 9).

One effect of hypophysectomy, as is well known, is an increase in the width of the z. intermedia and the z. glomerulosa. A major part of this change is undoubtedly due to the shrinkage in size of the adrenal (most marked in z. reticularis and z. fasciculata) which greatly reduced the surface area of the ellipsoid over which the peripheral zones are spread. Until the 3rd week after hypophysectomy the z. intermedia

still shows a greater number of cells per unit area than the other parts of the cortex, but later than this, shrinkage of cells throughout the cortex results in homogeneity of cell size in the outer three zones (Lever, 1954).

The changes following hypophysectomy plus castration are essentially those which follow hypophysectomy alone. Thus after 2 weeks, the adrenal of the *castrated* hypophysectomized rat appears identical with that of the hypophysectomized rat (Pl. 1, fig. 8).

In the hypophysectomized rat treated with ACTH, the changes in ascorbic acid distribution (see section C) can be demonstrated within the hour. Changes in the lipid distribution are more difficult to assess. Rats hypophysectomized 48 hr. previously showed a slight depletion of adrenal lipids  $4\frac{1}{2}$  and 8 hr. after high dosage with ACTH. Changes were not observed in the z. intermedia. Reiss *et al.* (1936-7) and Simpson *et al.* (1943) describe the re-establishment of the normal orderly arrangement of zones in the adrenal after treating hypophysectomized animals with ACTH. Cater & Stack-Dunne (1953), however, found that the adrenals of rats hypophysectomized 2 weeks previously and treated for 4 days with ACTH, or growth hormone, could be distinguished from the adrenals of normal rats. The glands from such treated animals do not show a sudanophobe zone in lipid preparations, nor is the z. intermedia apparent in haematoxylin and eosin preparations.

#### DISCUSSION

From the present findings a well-defined z. intermedia is apparently associated with a z. glomerulosa of regular form (columnar or rosette), while in adrenals where the z. glomerulosa is a diffuse cell collection, as in the cow (Pl. 1, fig. 10), the z. intermedia is ill defined. A possible suggestion is that the z. intermedia interposed as it is, between glomerulosa and fasciculata (each with their distinctive cell groupings), constitutes a region of cellular re-arrangement. Thus it would not be well defined in glands in which the cells of the outer layers of the cortex are diffusely grouped.

Cell zoning in the frog adrenal cortical tissue was originally suggested by Stilling (1898): in the cortical cell columns just internal to the rounded peripheral cells, he observed cell flattening at right angles to the column axis: he strikingly described these flat cells as 'stacked like logs'. At variance with both Stilling's description, and the present observations, is the account of the frog adrenal by Sluiter, Mighorst & van Oordt (1949); they refer only to one type of 'interrenal' cell which they claim is filled with lipid. Grynfeldt (1904) observed less lipid in the peripheral than in the deeper cortical cells of the frog adrenal; and Bulliard, Maillet & Droz (1953) reported that after ACTH treatment in the frog, lipid depletion was maximal in the peripheral cortical cells.

The presence within the cortical cell columns of the frog adrenal of a region of flattened cells and nuclei, akin to those of the mammalian z. intermedia, suggests that this zone is not only a property of the compact concentrically layered adrenal cortex. Mitchell (1948) claimed that the flattening of cells in the rat z. intermedia is due to compression, but he was not specific as to the cause of the compression. Cell flattening and smallness of cell size though usual, are nevertheless not invariable features of a z. intermedia, as already indicated. It is a significant fact that in the pregnant rat adrenal, in which the z. fasciculata is wider than normal, there is often



marked flattening of cells between the zonae glomerulosa and fasciculata (Pl. 1, figs. 3, 9). A hypertrophy of the z. fasciculata relative to the other zones is likely to increase the tension within the gland, and cell flattening may be an expression of this. Fluctuations of cell lipid content and hence cell size in the z. fasciculata of the normal rat adrenal have been claimed as physiological (Cain & Harrison, 1950). If this is true, then the presence of real cell flattening in the z. intermedia may be conditional on the degree of cellular distension in the z. fasciculata.

The view that an increased number of cells per unit area in the z. intermedia may be due to excessive mitotic activity within the zone, is probably untenable. Mitoses in the zone, though present, are few in number (Cater & Stack-Dunne, 1953). The possibility that the z. intermedia may be the product of rapid cell division at the inner edge of the z. glomerulosa or outer edge of the z. fasciculata must be considered, particularly as these are the regions of maximum mitotic activity in the cortex (Mitchell, 1948; Cater & Stack-Dunne, 1953). If an inward passage of cells from the z. glomerulosa is responsible for the presence of a z. intermedia, this would support the popular theory of a centripetal migration of cortical cells.

There is no doubt that the cells of the z. intermedia are subject to the influence of the anterior pituitary. It is interesting to note that when hypophysectomized rats are treated with ACTH the fat-free cells of the z. intermedia become filled with fat, and if treatment is continued they become indistinguishable from the lipid-laden z. fasciculata cells. In this connexion it should be restated that the z. intermedia is usually not clearly identifiable, either histologically or histochemically, in rat adrenals of both sexes between the 6th and 26th weeks (approximately). During this time the animal reaches sexual maturity. After the 26th week, and increasingly with old age, the zone is more clearly defined. It is suggested that the level of anterior pituitary activity is a factor controlling the presence or absence of cells constituting a z. intermedia. Greep & Jones (1950), observing the effects of androgens and oestrogens on the adrenals of intact and gonadectomized rats of both sexes, conclude that androgens probably favour the formation of a z. intermedia ('transitional zone') but are not an essential condition, since the zone is present in the adrenals of untreated spayed females.

The present findings on lipid distribution in the adrenals of the common domestic animals largely confirm those of Nicander (1952). A rigid claim that the z. intermedia is invariably fat-free or fat-filled in any animal is probably unwise, as our experiments on the rat have indicated. The presence or absence of lipid in the zone may depend, among other things, on the level of pituitary stimulation of the adrenal.

The z. intermedia, in normal adult male rats, constitutes a zone of capillary compression with a sharp outer edge corresponding to the inner limit of the z. glomerulosa (Lever, Cater & Stack-Dunne, 1953; Lever, 1954). Clearly this capillary compression is directly proportional to the degree of cell packing within the z. intermedia. In rats hypophysectomized several weeks previously, the adrenal cortex with a high degree of cell packing is much less vascular than normal. The question arises as to whether the action of the anterior pituitary on the cells of the outer layers of the cortex does in fact alter the degree of compression in the z. intermedia and thus exercise some control over the capillary blood flow. In this connexion it must be

mentioned that the *arteriae corticis* (Flint, 1900) arising from the subcapsular arterial plexus in the rat, most commonly join the basic capillary bed in the outer z. fasciculata and the z. glomerulosa (Gersh & Grollman, 1941; Lever, 1952). Fine nerves, with occasional boutons, have been described in relation to vessel walls in the outer layers of the rat adrenal cortex (Lever, 1952). It is probable that some, at any rate, of these nerves provide a vaso-motor control over the *arteriae corticis*. The suggestion is made that in the rat at any rate, sudden alterations in the blood flow through the outer layers of the cortex may be effected by alterations in the calibre of the *arteriae corticis*, while a basic and slower control is exercised by the degree of capillary compression within the z. intermedia and outer z. fasciculata. This last form of control is probably under the influence of the anterior pituitary.

#### SUMMARY

1. The comparative morphology of the adrenal cortex is described in certain Amphibia, reptiles, birds and common domestic and laboratory mammals, with special reference to the presence or absence of the z. intermedia.

(a) The frog adrenal shows some zoning; in other amphibians, reptiles and birds there is none.

(b) In the rat, cat, dog, sheep, rabbit and horse, in which the z. glomerulosa has a regular form, the z. intermedia is well defined. In the guinea-pig, mouse, cow and pig it is not readily seen. The zone is usually sudanophobe in the rat, dog and horse, but contains lipids in the other mammals studied.

2. In the developing rat's adrenal the z. intermedia is present by the 3rd day after birth. It is prominent and sudanophobe up to the 6th week, but between 6 weeks and 6 months it is less prominent and may contain lipids. After the 6th month it becomes increasingly prominent as the rat ages. At no stage of development is there any concentration of mitotic figures in the z. intermedia. The z. glomerulosa and the outer part of the z. fasciculata appear to be the chief sites of cell division. Between the 28th day and the 35th day mitotic activity becomes much reduced.

3. A histochemical study of the rat's adrenal does not suggest that the z. intermedia is a fixation artefact. The zone is usually free from lipids, and cholesterol, is not outlined by special phosphatase or esterase activity, but the cytoplasm of its cells is stained with pyronin. Ascorbic acid is still present in the cells of the z. intermedia 1 hr. after treatment with ACTH but ascorbic acid is leaving the cells of the z. fasciculata and entering the capillaries.

4. The changes in the z. intermedia are described after hypophysectomy, castration, and treatment with ACTH.

5. The role of z. intermedia in the adrenal cortex is discussed with special reference to the capillary circulation.

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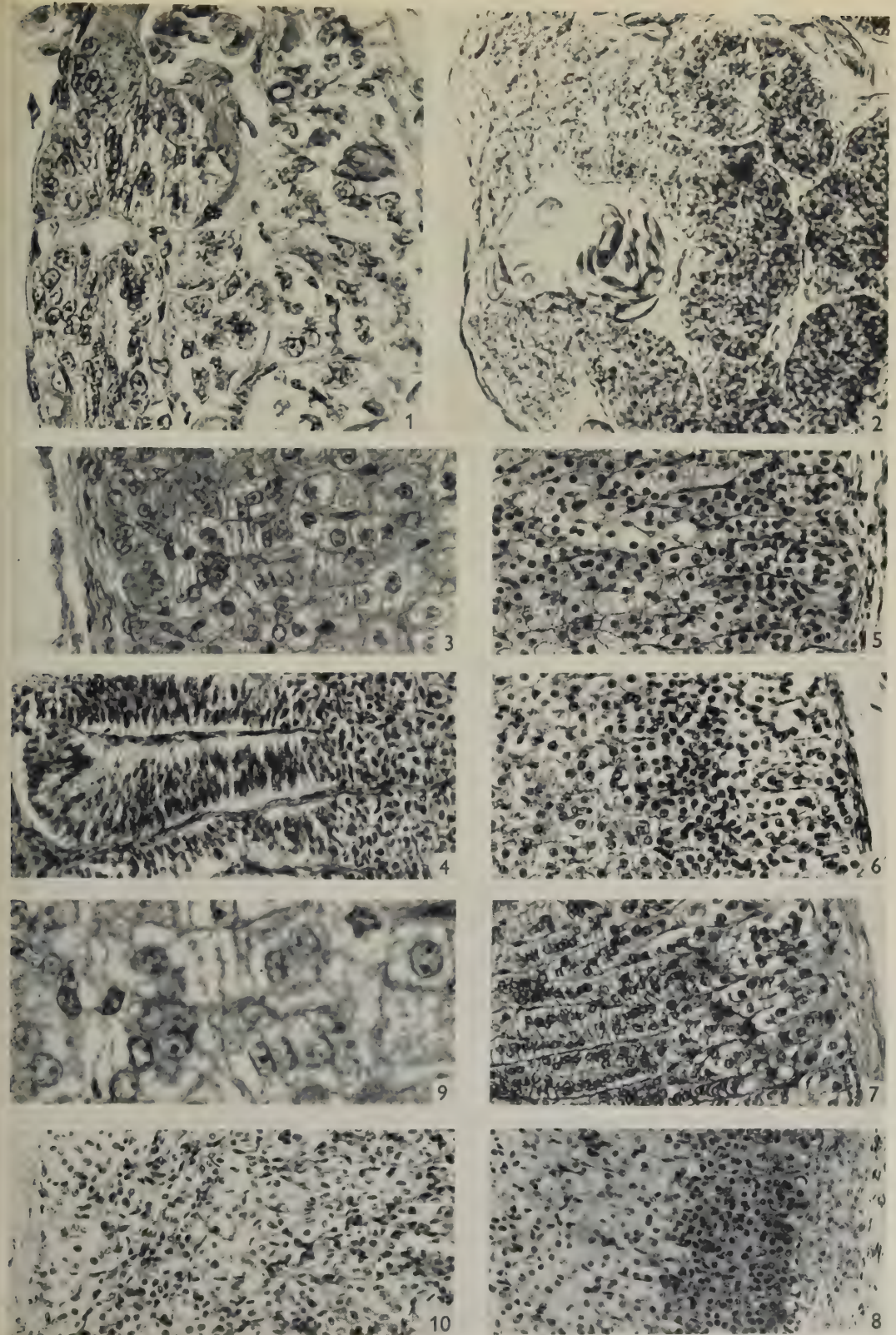


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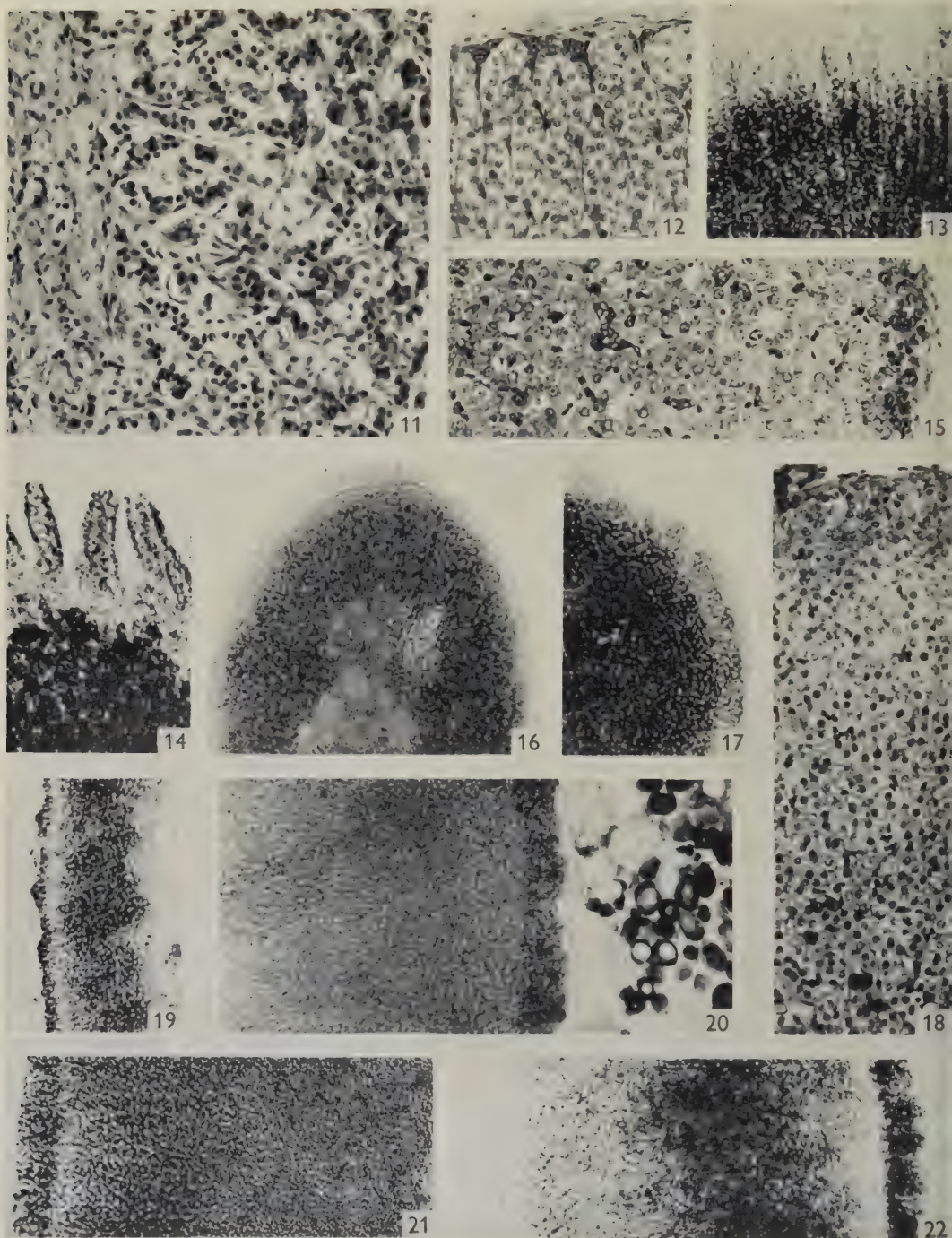
## EXPLANATION OF PLATES

## PLATE I

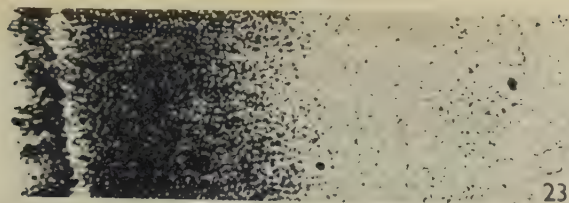
- Fig. 1. Frog adrenal: 7  $\mu$  haematoxylin and eosin preparation,  $\times 450$ . From left to right note: (i) the capsule; (ii) glomerulosa groups of cells with round nuclei; (iii) a layer of flattened cells with spindle-shaped nuclei; (iv) a spongiocyte layer of vacuolated cells. A few medullary cells are seen as darkly stained areas intermixed with the spongiocytes.
- Fig. 2. Frog adrenal: 25  $\mu$ , gelatine-embedded frozen section stained with Scharlach R,  $\times 450$ . Note major lipid distribution in spongiocyte layer (right), with outer part of cortical columns comparatively lipid-free. Refer to fig. 1.
- Fig. 3. Pregnant rat adrenal: 7  $\mu$  haematoxylin and eosin preparation,  $\times 230$ . From left to right note: (i) the capsule; (ii) the z. glomerulosa; (iii) a layer of flattened cells, some showing flattened nuclei; (iv) the spongiocytes of the outer z. fasciculata. Compare with fig. 1.
- Fig. 4. Horse adrenal: 10  $\mu$  haematoxylin and eosin preparation,  $\times 230$ . From left to right, note: (i) long arched columns of the z. glomerulosa with cells and nuclei at right angles to the long axis of column; (ii) the z. intermedia seen as a layer of cellular re-arrangement; (iii) the polygonal cells with rounded nuclei of the outer z. fasciculata.
- Fig. 5. Cat adrenal: 7  $\mu$  haematoxylin and eosin preparation,  $\times 230$ . From right to left, note: (i) the capsule; (ii) a rosette form of z. glomerulosa; (iii) the z. intermedia apparent as a layer of smaller packed cells; (iv) the spongiocytes of the outer z. fasciculata.
- Fig. 6. 12-month-old rat adrenal: 7  $\mu$  haematoxylin and eosin preparation,  $\times 230$ . From right to left, note: (i) the capsule; (ii) the z. glomerulosa of regular form; (iii) an increased number of cells per unit area is well seen in the z. intermedia; (iv) the spongiocytes of the z. fasciculata.



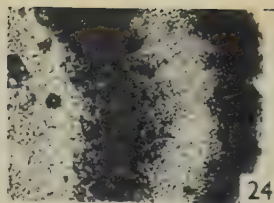




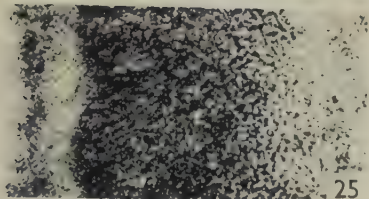




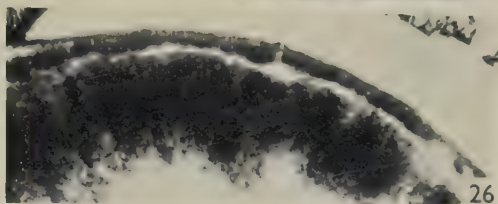
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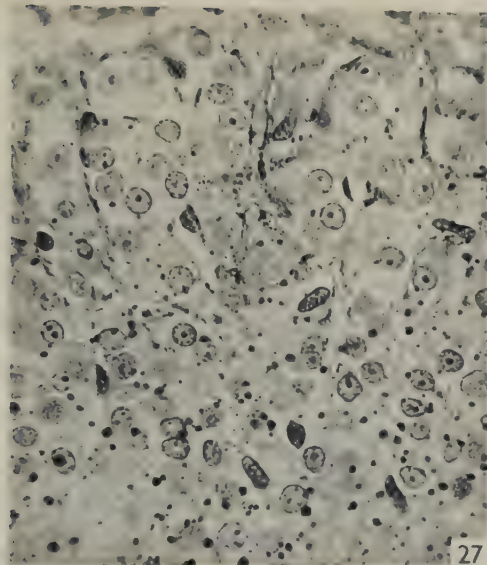
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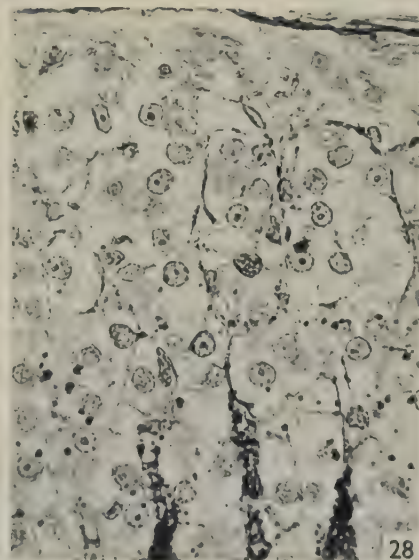
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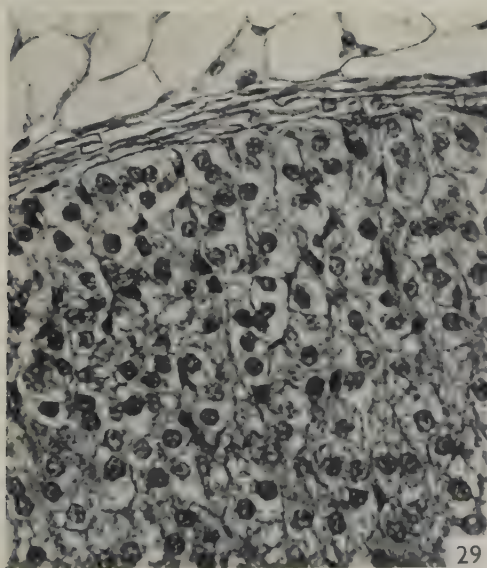
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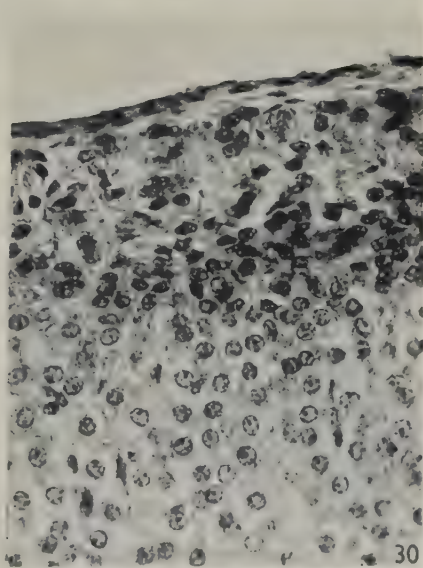
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- Fig. 7. Rabbit adrenal: 7  $\mu$  haematoxylin and eosin preparation,  $\times 230$ . From right to left, note: (i) a columnar z. glomerulosa; (ii) well-marked cell flattening and packing in z. intermedia which is broad and irregular in extent; (iii) the outer z. fasciculata.
- Fig. 8. Rat adrenal 2 weeks after hypophysectomy: 6  $\mu$  section,  $\times 230$ . Stained by the methyl green-pyronin method. Marked basophilia of the zonae glomerulosa and intermedia contrasts with the palely stained thickened capsule to the right, and the z. fasciculata to the left.
- Fig. 9. High-power view ( $\times 820$ ) of the pregnant rat adrenal in fig. 3. Note: (i) cell flattening in the z. intermedia; (ii) in some cells of the z. intermedia nuclei are flattened; in others the plane of section does not include a nucleus; (iii) the spongiocytes of the outer z. fasciculata are seen to the right.
- Fig. 10. Cow adrenal: 10  $\mu$  haematoxylin and eosin preparation,  $\times 230$ . From left to right, note: (i) the capsule; (ii) an irregular form to the z. glomerulosa; (iii) there are no very definite out-lines to the z. intermedia but differences in cell grouping suggest its presence; (iv) the outer z. fasciculata.

PLATE 2

- Fig. 11. Ox adrenal: 10  $\mu$  haematoxylin and eosin preparation,  $\times 230$ . From left to right, note: (i) the capsule; (ii) loose cell grouping in the z. glomerulosa; (iii) a z. intermedia of smaller cells with basophilic cytoplasm; (iv) the outermost z. fasciculata.
- Fig. 12. Mouse adrenal: 7  $\mu$  haematoxylin and eosin preparation,  $\times 230$ . From above downwards, note: (i) the thin capsule; (ii) rounded cell clusters in the z. glomerulosa; (iii) the z. intermedia is difficult to define although there is a suggestion of cell packing between the zonae glomerulosa and fasciculata.
- Fig. 13. Rabbit adrenal: 25  $\mu$  gelatine-embedded frozen section stained with Sudan black,  $\times 90$ . The zonae intermedia and fasciculata contain lipid while the z. glomerulosa, above, is almost lipid-free.
- Fig. 14. Horse adrenal: 25  $\mu$  gelatine-embedded frozen section stained with Sudan black,  $\times 90$ . A sudanophobe z. intermedia is interposed between a lipid-containing z. glomerulosa (above) and a lipid-laden z. fasciculata (below).
- Fig. 15. Adrenal of a 1.8 g. rat embryo: 6  $\mu$  haematoxylin and eosin preparation,  $\times 230$ . From right to left, note: (i) a thin cellular capsule with a layer of dark-staining cells immediately deep to it; (ii) a broader zone of vacuolated pale cells which in frozen preparations contain lipid; (iii) the large lipid-free eosinophilic cells of the foetal cortex. Within the foetal cortex is an irregular darkly stained clump of chromaffin (medullary) cells.
- Fig. 16. Adrenal of a 1 g. rat embryo: 25  $\mu$  gelatine-embedded frozen section stained with Sudan black,  $\times 90$ . Two major regions are seen: (i) an outer zone consisting of the lipid-containing anlage of the adult cortex; and (ii) an inner patchy zone with little or no lipid staining, comprising the foetal cortex (see fig. 15).
- Fig. 17. Adrenal of a 3-day rat: 25  $\mu$  gelatine-embedded frozen section stained with Sudan black,  $\times 90$ . The first signs of a fat-free z. intermedia appear at this age. The outer edge of the lipid laden z. fasciculata marks its inner limit. See fig. 18.
- Fig. 18. Adrenal of a 3-day rat: 6  $\mu$  haematoxylin and eosin preparation,  $\times 230$ . From above downwards note: (i) the capsule and deep to it an outer darkly stained zone which is differentiating into z. glomerulosa and z. intermedia. In the z. intermedia an increased number of cells per unit area is noticeable; (ii) the z. fasciculata comprises the remainder of the cortex; the pale spongy cells of the outer z. fasciculata are lipid-laden in frozen preparations.
- Fig. 19. Adrenal of 10-day rat: 25  $\mu$  gelatine-embedded frozen section stained with Sudan black,  $\times 90$ . From left to right, note: (i) the lipid-containing z. glomerulosa; (ii) a lipid-free z. intermedia; (iii) the outer z. fasciculata is lipid-laden, while the inner part of the z. fasciculata, bordering the unstained medulla, is almost fat-free.
- Fig. 20. Adrenal of a 4-month rat: 25  $\mu$  gelatine-embedded, frozen section, stained with Sudan black,  $\times 90$ . The lipid-laden z. glomerulosa, on the right, contrasts with the fine lipid staining in the zonae intermedia and fasciculata.
- Fig. 21. Adrenal of a 9-month rat: 25  $\mu$  gelatine-embedded frozen section, stained with Sudan black,  $\times 90$ . Note the fat-free z. intermedia, or sudanophobe zone. Compare the lipid distribution with that in fig. 20.



Fig. 22. Adrenal of a 1-year rat: 25  $\mu$  gelatine-embedded frozen section stained with Sudan black,  $\times 90$ . The *z. intermedia* is seen as a wide sudanophobe layer internal to the lipid-laden *z. glomerulosa* on the right.

## PLATE 3

Fig. 23. Adrenal of rat hypophysectomized 24 hr. previously: 25  $\mu$ , frozen section, stained by the Schultz method,  $\times 90$ . The *z. intermedia* stands out as a clear band between the Schultz-positive zonae glomerulosa and fasciculata.

Fig. 24. Schultz preparation (as for fig. 23) of adrenal of rat hypophysectomized 3 weeks previously,  $\times 90$ . The *z. intermedia*, unstained, is broader than in fig. 23.

Fig. 25. Adrenal of rat hypophysectomized 2 weeks previously: 25  $\mu$  frozen section, stained by the Baker acid haematein method,  $\times 90$ . From left to right note: (i) an irregularly stained *z. glomerulosa*; (ii) an unstained *z. intermedia*; (iii) a heavily stained outer *z. fasciculata*; (iv) the inner fasciculata and the reticularis contain small amounts of stained material.

Fig. 26. Fresh frozen section at 25  $\mu$  of rat adrenal, stained intravitaly by methylene blue,  $\times 25$ . The photograph was taken 5 min. after death. From above downwards, note: (i) stained capsule and *z. glomerulosa*; (ii) a thin but definite unstained *z. intermedia*; (iii) a stained *z. fasciculata*; (iv) a narrow patchily stained *z. reticularis* bordering on an unstained medulla.

Figs. 27 and 28. From 6  $\mu$  sections of rat adrenals,  $\times 450$ ; ascorbic acid is demonstrated as black granules by the acetic acid-silver nitrate method, and nuclei are counterstained with neutral red. Fig. 27 (the control) shows the ascorbic distribution in the left adrenal removed from a rat 24 hr. after hypophysectomy. In the upper part of the print, the ascorbic acid content in the deep layers of the capsule and the *z. glomerulosa*, is low, while internal to this in the *z. intermedia*, the granules are larger and more numerous. The outermost cells of the *z. fasciculata* with large silver granules are seen at the bottom of the print. The silver granules do not noticeably lie within vessel walls. Fig. 28 shows the ascorbic distribution in the right adrenal of the same rat 1 hr. after a medium dose of ACTH (Armour, 84-85 H. 0.1  $\mu$ g. intravenously). Cell depletion of ascorbic acid silver granules in the outer *z. fasciculata* is associated with a crowding of these into the vessel lumina. At this stage (1 hr. after ACTH) some silver granules are still present in the *z. intermedia*.

Fig. 29. 2-month-old male rat adrenal: 7  $\mu$  haematoxylin and eosin preparation,  $\times 400$ . There is no obvious *z. intermedia* between glomerulosa and fasciculata at this age.

Fig. 30. 2½-year-old rat adrenal: 7  $\mu$  haematoxylin and eosin preparation,  $\times 400$ . A very well defined and basophilic *z. intermedia* exhibits marked cellular and nuclear flattening, which is also a feature of the deeper glomerulosa cells; the *z. glomerulosa* is patchily basophilic.

# POST-NATAL FATE OF THE ABDOMINAL PARA-AORTIC BODIES IN MAN

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## INTRODUCTION

It is now generally accepted that the chromaffin cells of the human foetus or young child are to be found both inside the adrenal gland and in the retroperitoneal tissues closely associated with the prevertebral sympathetic plexuses. The extra-adrenal collections are known as sympathetic paraganglia or para-aortic bodies.

Both intra-adrenal and extra-adrenal chromaffin cells are developed from cells of the primitive sympathetic anlage (Kohn, 1903; Iwanow, 1930, 1932; Coupland, 1952), but whilst it is generally recognized that the adrenal medulla persists throughout life, the subsequent fate of the para-aortic bodies is less well understood. In earlier investigations attention has invariably been focused primarily on the largest individual collection of chromaffin cells which are to be found close to the origin of the inferior mesenteric artery—the organs of Zuckerkandl. In the present work the writer has attempted to follow the fate of the extra-adrenal chromaffin tissue as a whole, from birth to adult life.

## HISTORICAL

Relatively little work has been done on the post-natal fate of the para-aortic bodies.

Bonnamour & Pinatelle (1902) observed an apparently normal body in a 6-year-old child, but failed to find extra-adrenal chromaffin cells in the adult. Zuckerkandl (1912) reported hyaline degeneration of these structures in a 2-year-old child and a 15-year-old youth; in specimens obtained from subjects aged 19 and 39 years, microscopic collections of chromaffin cells were observed 'in regions formerly occupied by them'; the actual site was not indicated.

Lucas Keene & Hewer (1927) reported fibrous degeneration in the bodies of a new born child, but did not continue the investigation into older post-natal specimens.

The most extensive investigations into the post-natal fate of the para-aortic bodies were undertaken by Ivanoff (1925) and Iwanow (1930); these authors concentrated primarily on the organs of Zuckerkandl, and concluded that maximum development was reached at about 2 years of age with subsequent degeneration. Degenerative changes described included hyperaemia, lymphoid infiltration, nuclear pyknosis and irregularity, vacuolation and hyalinization of cytoplasm. The final stage was said to be one of fibrosis. Iwanow (1930) found that the degenerative changes were associated with a gradual diminution in the size of the organs of Zuckerkandl. An extensive review of the pre- and post-natal human adrenal gland and extra-adrenal chromaffin tissue was published by Iwanow (1932); this included

a discussion on the physiology and pathology of these structures. On the subject of the post-natal fate of the para-aortic bodies no new facts were added to his previously published observations.

#### MATERIAL AND METHODS

Material used included two infants aged 5 months and 18 months; other specimens were aged 3, 5, 6, 7, 14, 22, 41 and 49 years. Tissues were obtained as soon as possible after death (2-12 hr.). The abdominal aorta and surrounding tissues were removed *en masse* and fixed by immersion in formol-dichromate (neutral formaldehyde 5%, potassium dichromate 3%) for 24 hr.; in the older specimens fixation was completed by immersing in 5% formaldehyde for a further 2 days. One specimen (3 years) was fixed by immersing in formol-potassium iodate. Specimens were cut into suitable portions, dehydrated and embedded in paraffin wax, serial sections were made and 1 in 4 to 1 in 10 (depending upon the size of the specimen) mounted. Alternate slides were stained routinely by Ehrlich's haematoxylin and Giemsa, the Giemsa being differentiated in an acid medium (Coupland, 1954). A few slides were stained with haematoxylin and eosin and iron haematoxylin. Graphic reconstructions were made.

#### RESULTS

##### *5-month-old infant*

Death resulted from broncho-pneumonia.

Para-aortic bodies are numerous (Text-fig. 1) and are associated with the pre-vertebral sympathetic plexuses. Chromaffin cells are arranged in cords alongside capillary blood vessels, the whole being surrounded by a fibrous capsule (Pl. 1, fig. 2). Nerve fibres are observed traversing sections of the larger bodies. The general appearance is similar to that of the older foetus (Pl. 1, fig. 1). Mitotic figures are not observed. One small body is seen in apposition with a lymph node but the capsule is intact and there is no evidence of lymphoid infiltration.

The organs of Zuckerkandl have a maximum length of 9 mm. and are not united by an isthmus.

##### *18 months*

Death resulted from pneumonia which complicated diabetes mellitus.

When received, this specimen had less peri-aortic tissue than the previous one, a fact which probably accounts for the apparent reduction in the number of bodies as compared with the previous specimen (Text-fig. 2). The para-aortic bodies are again scattered throughout the pre-vertebral sympathetic plexuses and have a late foetal appearance. There is no evidence of degeneration or cellular infiltration. The organs of Zuckerkandl have a maximum length of 12 mm. and are not united by an isthmus. Mitotic figures are not observed.

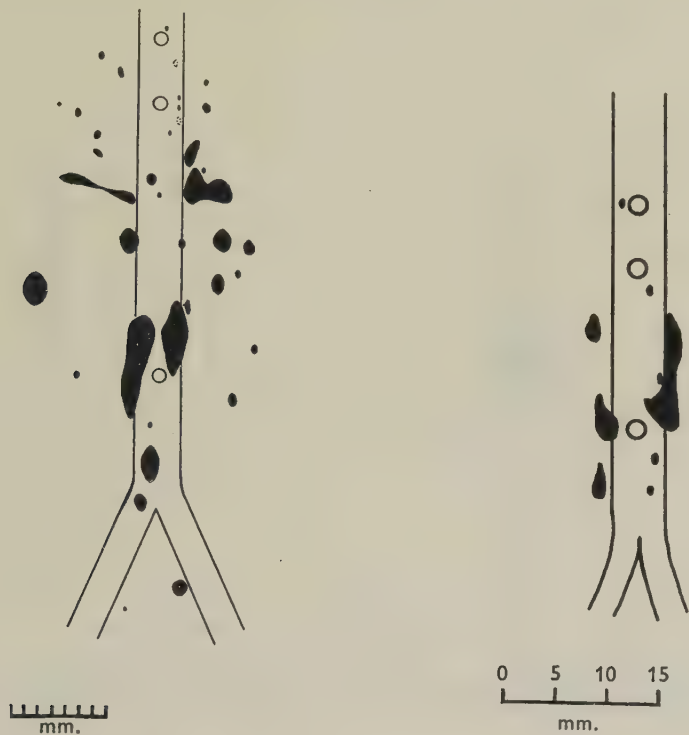
##### *3 years*

Death followed operation for congenital heart disease (Fallot's tetralogy).

Para-aortic bodies are numerous (Text-fig. 3) and resemble those of earlier specimens (Pl. 1, fig. 3). The organs of Zuckerkandl are larger than those of any earlier specimen and have a maximum length of 20 mm. A process extends across the midline from the left body but the two are not structurally continuous.



The chromaffin cells of the adrenal medulla and para-aortic bodies appear identical after staining with Ehrlich's haematoxylin, iron haematoxylin and Giemsa. The nuclei are rounded with a diameter of  $6-9\mu$  and contain scattered chromatin granules. The cytoplasm is faintly granular but the chromaffin reaction is poor as formol-iodate was used as a fixative; formol-iodate is much inferior to formol-dichromate in producing adrenochrome (Coupland, 1954). No degenerative



Text-fig. 1.

Text-fig. 2.

Text-fig. 1. Reconstruction of the abdominal aorta and surrounding tissues of a 5-month-old child. The sites of origin of the coeliac, superior mesenteric and inferior mesenteric arteries are indicated. Para-aortic bodies black.

Text-fig. 2. Reconstruction of an 18-month-old child. Para-aortic bodies black.

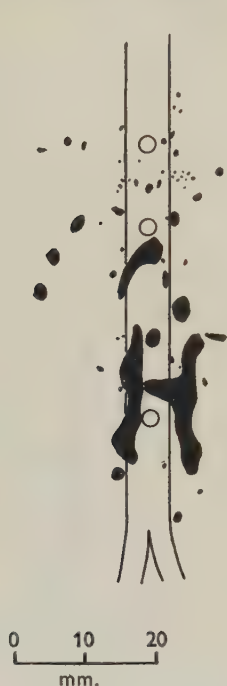
changes are present, nor is there evidence of cellular infiltration or of an obvious increase in connective tissue stroma. In the region between the superior and inferior mesenteric arteries a small body is observed lying in the centre of a lymph-node; it has an intact capsule and there is no evidence of lymphoid infiltration (Pl. 1, fig. 4).

### 5 years

Cause of death was miliary tuberculosis.

When received, the rostral part of the specimen was relatively denuded of peri-aortic tissue; this fact probably accounts for the small number of para-aortic bodies in the region of the superior mesenteric artery and coeliac axis (Text-fig. 4).

The organs of Zuckerkandl are now longer than in any previous specimen but, when compared with the organs of the younger child and foetus, have a definitely abnormal appearance. The most striking feature is an increase in the amount of connective tissue stroma and in peri-arterial fibrous tissue (Pl. 2, fig. 5); these changes affect all bodies. Blood vessels of nearby lymph-nodes also show a similar peri-



Text-fig. 3. Reconstruction of a 3-year-old child. Para-aortic bodies black.



Text-fig. 4. Reconstruction of a 5-year-old child. Para-aortic bodies black.

arterial increase in fibrous tissue, and it is likely that this is a general change and not one confined to the para-aortic bodies. There is no evidence of endarteritis. In the specimen many of the chromaffin cells are vacuolated, as are many of the nearby sympathetic neurones, and it is concluded that this is either a toxic or post-mortem change. Lymphoid infiltration is not observed.

#### *6 years*

Death resulted from a cerebral tumour.

All the para-aortic bodies show some increase in connective tissue stroma and peri-arterial fibrous tissue, but again the latter change can be observed in nearby blood vessels which are not connected with the para-aortic bodies. There is no evidence of endarteritis. Apart from these changes the para-aortic bodies in relation to the upper part of the aorta have a normal appearance. The organs of Zuckerkandl have undergone further changes and are now virtually unrecognizable as definite structures; instead small groups of chromaffin cells associated with nerve fibres and

vascular connective tissue (capillaries being numerous) extend down in association with the pre-aortic plexus on each side of the inferior mesenteric arteries. There is no evidence of lymphoid infiltration.

#### *7 years*

Death was due to a cerebral abscess.

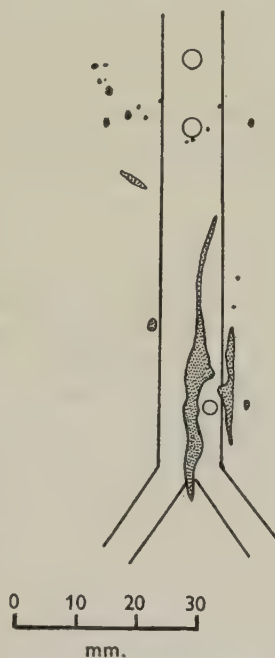
As in the previous specimen, the organs of Zuckerkindl no longer exist as distinct entities, and instead small collections of chromaffin cells associated with vascular connective tissues and nerve fibres of the pre-aortic plexus extend down on each side of the inferior mesenteric artery (Pl. 2, fig. 6). In comparison with earlier specimens there has been a definite reduction in the number of chromaffin cells present in any one transverse section of the region formerly occupied by the organs of Zuckerkindl. Chromaffin cells extend further in a cranio-caudal direction (Text-fig. 5): a finding which suggests distraction of the organs. Collections of chromaffin cells in the vicinity of the superior mesenteric and coeliac arteries (Pl. 2, fig. 7) resemble more closely the bodies of the earlier post-natal specimens, but differ in having a more abundant stroma; the larger bodies are also more irregular and in this respect resemble the remains of the organs of Zuckerkindl. There is no evidence of lymphoid infiltration.

#### *14, 22, 41 and 49 years*

Deaths were due respectively to appendicitis (post-operative), subarachnoid haemorrhage, mitral heart disease and coronary thrombosis.

In each case the findings are similar to those reported for the 7-year-old specimen (Text-fig. 5).

In the neighbourhood of the superior mesenteric artery two to four discrete para-aortic bodies have been found in all specimens; these usually differ from the late foetal bodies in that the connective tissue stroma is more abundant and that they are less regular in shape (Pl. 2, fig. 8). However, one rostral body of the 41-year-old subject has a close resemblance to those of the young child (Pl. 2, fig. 9). In the 14-year-old specimen the bodies are engorged with blood, a condition which probably resulted from the disease process. A few fat cells are associated with one of the larger bodies (diameter 1.6 mm.) found in the 49-year-old specimen. Lymphoid infiltration is not observed. In the caudal part of the specimens discrete bodies are not found but a few scattered chromaffin cells are associated with pre-aortic sympathetic nerve fibres and extend down on each side of the inferior mesenteric artery; they are usually associated with vascular connective tissue. The findings are very similar to those reported for the 7-year-old specimen; the discrete rostral bodies have a similar position and differ structurally only in the further increase in stroma, which may



Text-fig. 5. Reconstruction of a 7-year-old child. Encapsulated para-aortic bodies black; the positions of the scattered collections of chromaffin cells are indicated by stippling.



include fat cells, whilst in the caudal part of the specimen scattered chromaffin cells are found in the area indicated in Text-fig. 5. The large size of the adult specimens and scattering of cells makes difficult a quantitative estimation and subsequent comparison with the young child. The writer is, however, of the opinion that in the period between 7 years and adult life there is a considerable reduction in the number of chromaffin cells in the caudal half of the pre-aortic region. The reduction in chromaffin tissue in the rostral portion of the specimens, if any, is much less marked. In a preliminary communication on this subject (Coupland, 1953*b*), the writer stated that extra-adrenal chromaffin cells had not been observed in a 49-year-old specimen. It is now apparent that this failure to find the cells was due to the examination of an insufficient amount of peri-aortic tissue, and the further examination of other parts of the same specimen has revealed the presence of discrete collections of chromaffin cells. It is necessary to section all the retroperitoneal tissues which lie anterior and immediately lateral to the aorta, plus the aorta itself if the bodies are to be found.

#### DISCUSSION

The results of the present investigation differ from those of previous works in showing that a considerable number of extra-adrenal chromaffin cells is present in the adult. Earlier workers either failed to find extra-adrenal cells in the adult (Bonnamour & Pinatelle, 1902) or found only isolated small collections (Zuckerkindl, 1912; Ivanoff, 1925; Iwanow, 1930, 1932). The failure of these earlier workers to find greater numbers of extra-adrenal cells is probably due to the fact that their attention was focused mainly on the organs of Zuckerkindl, which, as distinct entities, disappear during childhood.

The organs of Zuckerkindl undergo very marked changes during early post-natal life (Pls. 1 and 2, figs. 1-6) which have in the past always been referred to as degenerative: a term which, for the want of a better alternative, has been retained in this work. According to Ivanoff (1925), the change takes place in three stages, the first being one of hyperaemia, the second lymphoid infiltration and the third fibrosis; these changes were said to be associated with a concomitant change in the chromaffin cells, nuclear pyknosis or irregularity, and vacuolation or hyalinization of the cytoplasm.

During the present investigation, an increase in stroma has been observed in all para-aortic bodies obtained from specimens of and above 5 years of age; this increase is often most obvious in the perivascular region, but is in fact a diffuse change affecting the whole structure. In specimens aged 6 and 14 years, some of the bodies were engorged with blood, a condition described by Ivanoff (1925) as 'hyperaemia' and regarded by him as being a sign of degeneration. In the present series this engorgement has been an inconsistent finding and is most probably due either to the disease process present before death or to agonal changes. In at least one of the cases in which Ivanoff (1925) observed lymphoid infiltration, death resulted from caseous pneumonia (possibly tuberculosis), and it is conceivable that the infiltration was a pathological change. In the present series lymphoid infiltration has never been observed. When post-mortem material is used the cellular changes of the type described by Ivanoff (1925) and Iwanow (1930, 1932) are without significance

because nuclear pyknosis, vacuolation, hyalinization or 'cloudy swelling' of the cytoplasm is commonly present in greater or lesser degree not only in chromaffin cells but in other glandular organs and even in the sympathetic neurones; the changes being due to a combination of ante-mortem toxæmia and post-mortem degeneration. In the present work only the more permanent changes in the structure of the para-aortic bodies, viz. an increase in connective tissue stroma and gross irregularity of shape have been used as criteria for assessing degeneration. In spite of the difference in methods of assessing degeneration in the present investigation and in the works of Ivanoff (1925) and Iwanow (1930), there is a close correspondence in the estimation of the time at which the extra-adrenal chromaffin tissue reaches maximal development, i.e. 3 and 2 years respectively. Degenerative changes then supervene.

Iwanow (1930) considered that the isthmus form was typical of the fully developed organ of Zuckerkandl, and that absence of an isthmus in a child over the age of 2 years was a sign of early degeneration. As reported previously (Coupland, 1952), the writer considers the isthmus form to be an accidental occurrence and in no way specific. The findings during the present work support this view.

The para-aortic bodies increase in size throughout foetal life (Coupland, 1952), and continue to grow up to the age of 3 years. Nerve fibres are always closely associated with the bodies and may be observed passing through them. As the para-aortic bodies undergo a gradual change in structure throughout foetal and post-natal life, it is impossible to say at which stage they have a 'normal' appearance. In early foetal life the bodies contain primitive sympathetic cells as well as chromaffin cells and stroma; in late foetal life only chromaffin cells and supporting tissues are present; in post-natal life there is a gradual increase in the stroma which becomes very obvious after 3 years. At 5 years all these bodies show a marked increase in their fibrous tissue content; an increase in perivascular connective tissue is also observed but is not confined to the para-aortic bodies as it affects small arteries in all parts of the specimen, and it is not considered to be a factor specifically associated with the break up of the organs of Zuckerkandl. In the same specimen the organs of Zuckerkandl are becoming elongated and there is a definite reduction in the number of chromaffin cells present in any one section of these organs (as compared with earlier specimens). At 7 years and in all older specimens the more rostral bodies are still recognizable as distinct encapsulated units, but differ from earlier specimens in the greater amount of stroma, and, in some cases, the irregularity of shape. The chromaffin cells of the organs of Zuckerkandl no longer form circumscribed units but, instead, form small groups scattered along fibres of the pre-aortic sympathetic plexus.

It is difficult to estimate the total bulk of the extra-adrenal chromaffin tissue in the adult, but present findings indicate that in the rostral part of the specimen it approximates to that present in the young child, whilst in the lower pre-aortic region there is a definite reduction after the age of 7 years. The reduction may either be true—the result of cell death—or apparent—the result of the movement of chromaffin cells from the immediate vicinity of the aorta into the offshoots of the pre-aortic plexus. Examination of the proximal  $1\frac{1}{2}$  in. of the inferior mesenteric plexus and the upper part of the hypogastric plexus in adult specimens failed to

reveal the presence of chromaffin cells, and it would appear that a true reduction is involved.

During pre-natal life the extra-adrenal chromaffin tissue is precocious in development; present work indicates that it is at a maximum at 3 years of age. The early cessation of growth in chromaffin tissue is not confined to man, as Elliott & Tuckett (1906) found little increase in the size of the rabbit's adrenal medulla after the animal had reached 900 g. weight, and that the medulla of the 15-day-old guinea-pig had already attained adult proportions.

The fact that the elongated organs of Zuckerkindl are more markedly involved in the so-called degenerative changes which result in a subsequent disintegration of these structures, whilst the more circumscribed rostral bodies are less affected, suggests that the change is due to some local rather than a systemic influence. In the 3-year-old child the length of the abdominal aorta from coeliac axis to bifurcation in the formalin-fixed preparation is 5 cm., at 5 years this length has increased to 6 cm., at 7 years to 6½ cm., and in the adult is approximately 12 cm. As the aorta increases in length the pre-aortic nerve fibres are increasing in girth and length, and it is likely that the differential growth rate between the fully developed organs of Zuckerkindl and these nerve fibres results in the break up of the bodies and dispersal of chromaffin cells. Encapsulated collections of chromaffin cells can be observed in the 46 mm. human foetus (Coupland, 1952) and gradually increases in size up to the age of 3 years. Since the chromaffin cells of the human foetus and post-natal specimens appear identical after Bouin or formol-dichromate fixation and staining with haematoxylin or Giemsa or iron haematoxylin, and because extracts of foetal and neo-natal para-aortic bodies contain a pressor principle (West, Shepherd & Hunter, 1951; Coupland, 1953*a*), it appears reasonable to suppose that the chromaffin cells are mature and functionally active during this period. Goormaghtigh (1935) reported the post-natal migration of chromaffin cells in the mouse. It is, however, unlikely that in man a true migration of apparently mature and functionally active chromaffin cells occurs at this late stage. The fact that the major effect of differential growth is confined to the bodies in the pre-aortic region is explained by the close association between nerve fibres and the long axis of the bodies, whereas the small more circumscribed bodies found around the coeliac and superior mesenteric arteries, which have a maximum diameter of 3 mm. and are usually smaller, are less commonly traversed by obvious nerve fibres. Endarteritis has not been observed. Microscopic extra-adrenal collections of chromaffin cells have been observed in the adult by Zuckerkindl (1912), Ivanoff (1925) and Iwanow (1930, 1932), but these authors do not state the frequency with which such collections are found. In the present series extra-adrenal chromaffin cells have been found in all the adult specimens examined and appear to be a constant feature.

The general disposition of the extra-adrenal chromaffin tissue in the child and adult can be correlated with the site of formation of phaeochromocytomata. MacKeith (1944), reviewing the literature on this type of tumour, found that in 165 cases, 152 had involved the adrenal medulla whilst 13 had arisen in the retroperitoneal tissues between the kidneys.

The close association between lymphoid tissue and chromaffin tissue previously reported in the foetus (Coupland, 1952) has again been noted in post-natal specimens.



This either takes the form of side-to-side apposition of lymph node and para-aortic body or, less commonly, a small body may be seen lying in the centre of a lymph node; in both cases the capsule of the bodies is intact and there is no evidence of lymphoid infiltration. Lymphoid infiltration has not been observed in any para-aortic bodies. It is, therefore, concluded that the relation is fortuitous; this conclusion is supported by the relatively frequent finding of sympathetic nerve fibres in close apposition to or actually running through the centre of lymph-nodes.

#### SUMMARY

The abdominal para-aortic bodies increase in size up to the age of 3 years. Between 3 and 5 years so-called degenerative changes supervene in all bodies and are at first characterized by an increase in stroma. The changes are most marked in the organs of Zuckerkandl, which become elongated and eventually disintegrate. This process of disintegration can be observed in the child of 6–7 years of age and is complete by 14 years.

In the older child and adult the rostral bodies show an increase in stroma and in some cases irregularity, but disintegration is not usually observed.

In the adult discrete para-aortic bodies exist in the vicinity of the coeliac and superior mesenteric arteries, whilst only small microscopic collections of chromaffin cells are associated with the lower pre-aortic plexus.

Degenerative changes are not associated with lymphoid infiltration and the occasional juxtaposition of chromaffin and lymphoid tissue appears to be fortuitous.

I wish to thank Prof. A. Durward for his constant encouragement and advice, Dr W. K. J. Walls for producing the photographs, and Mr R. K. Adkin for assistance with the histological material.

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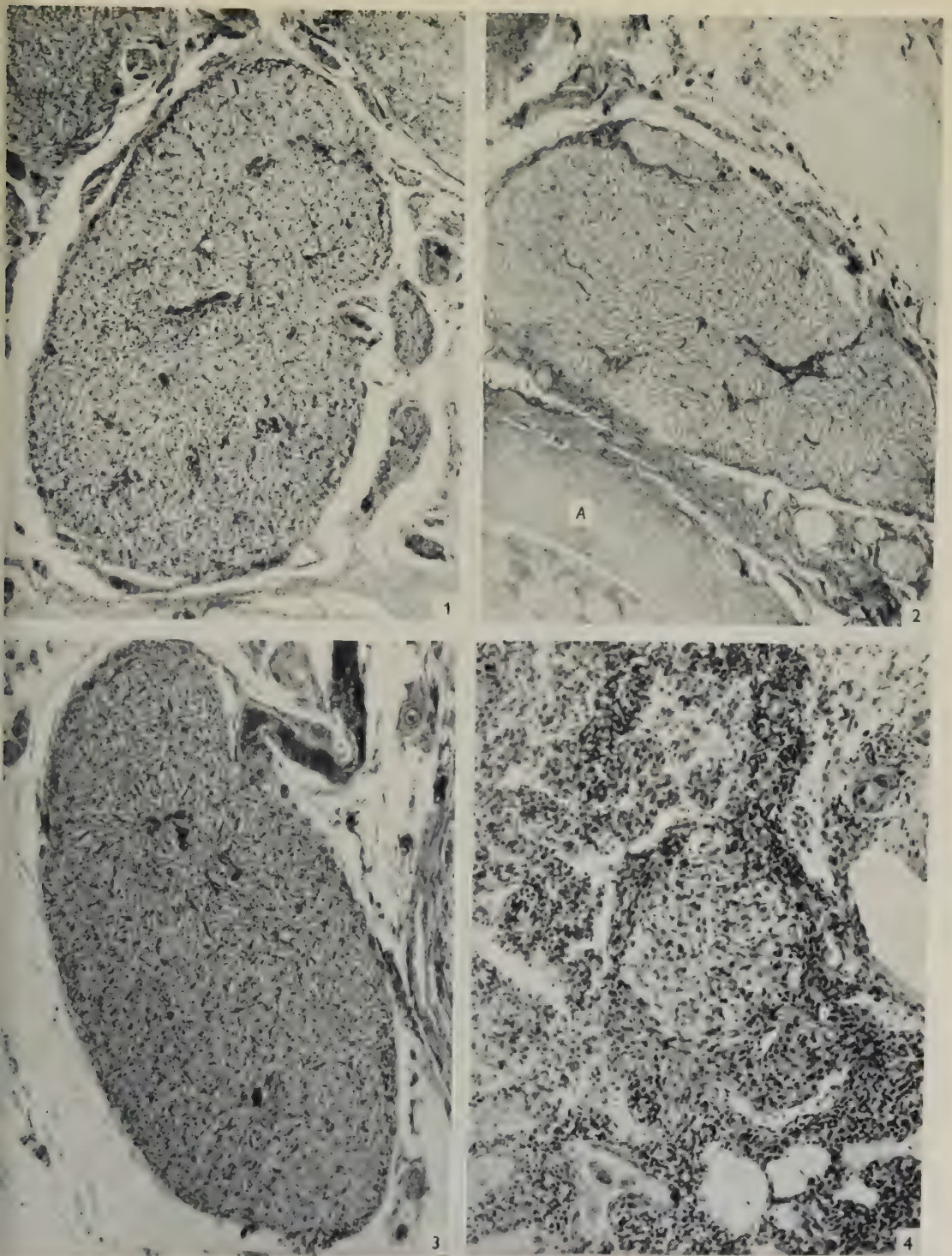
## EXPLANATION OF PLATES

## PLATE 1

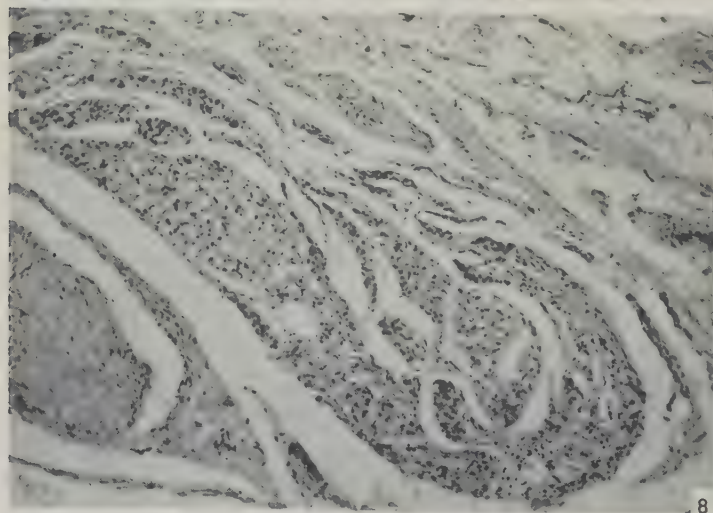
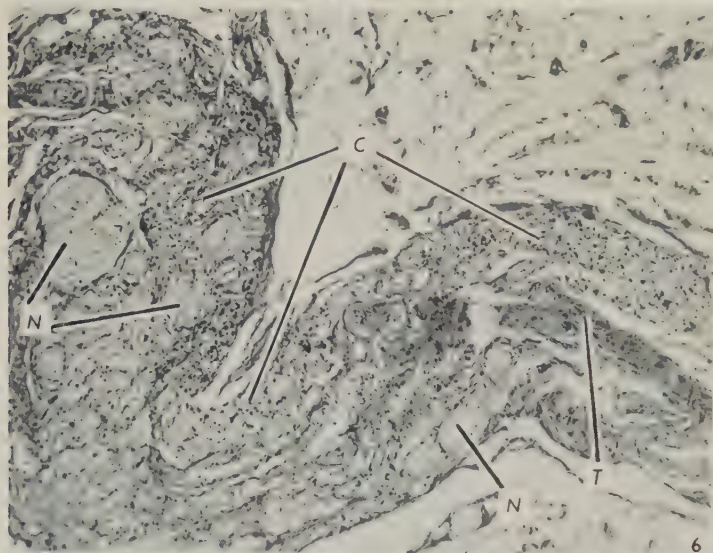
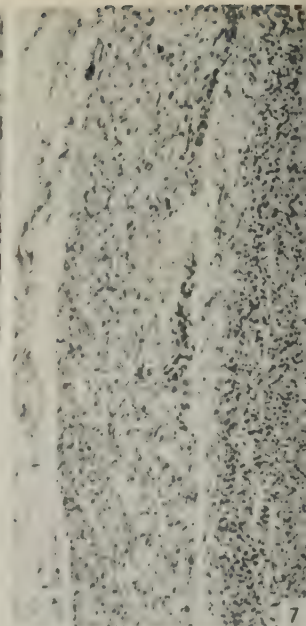
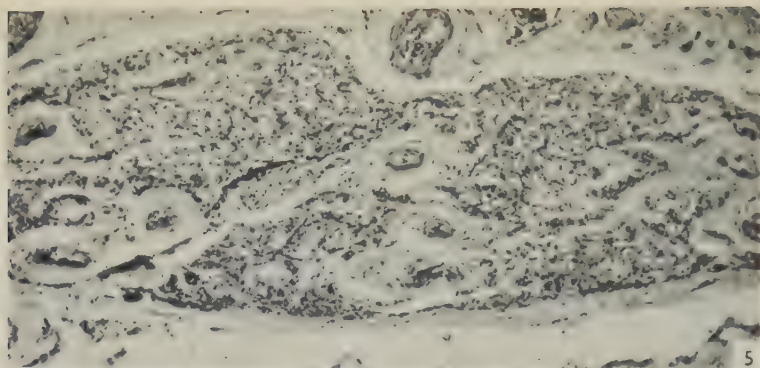
- Fig. 1. Encapsulated para-aortic body from the lower pre-aortic region of a 3 lb. 10 oz. premature infant. Haematoxylin and eosin. ( $\times 50$ .)
- Fig. 2. Section through an organ of Zuckerkandl of a 5-month-old infant. *A* = aorta. Haematoxylin and eosin. ( $\times 38$ .)
- Fig. 3. Para-aortic body from the lower pre-aortic region of a 3-year-old child. Haematoxylin and eosin. ( $\times 32$ .)
- Fig. 4. 3-year-old child. An encapsulated collection of chromaffin cells is lying inside a lymph node. Haematoxylin and eosin. ( $\times 122$ .)

## PLATE 2

- Fig. 5. Section of an organ of Zuckerkandl of a 5-year-old child. There is a marked increase in perivascular connective tissue. Iron haematoxylin. ( $\times 80$ .)
- Fig. 6. Section through an organ of Zuckerkandl of a 7-year-old child. Chromaffin cells (*C*) no longer form circumscribed bodies but are dispersed amongst nerve fibres (*N*) and connective tissue (*T*). Giemsa. ( $\times 86$ .)
- Fig. 7. Para-aortic body from the coeliac plexus of a 7-year-old child (left) in contact with a lymph node (right). Haematoxylin. ( $\times 110$ .)
- Fig. 8. A somewhat distorted but intact para-aortic body from the vicinity of the coeliac artery of a 41-year-old adult. Haematoxylin. ( $\times 80$ .)
- Fig. 9. Para-aortic body from the region of the superior mesenteric artery of a 41-year-old adult. This closely resembles the bodies of the young child. Haematoxylin. ( $\times 90$ .)







# REGENERATION OF NON-MEDULLATED NERVE FIBRES

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## INTRODUCTION

Return of function following nerve injury involves the downgrowth of axonal sprouts to re-establish contact with the denervated end-organs. The efficiency of the process depends not only on the ability of the axons to grow peripherally but also upon the integrity of the guiding nerve sheaths, which ensure that suitable contacts are made with the effector cells.

Numerous workers experimenting with somatic medullated nerves have shown that the degree of return of function depends, to a large extent, on the nature of the nerve injury (Seddon, 1943; Denny-Brown & Brenner, 1944; Young, 1949). When the nerve is crushed the axons are interrupted, but the connective tissue sheaths may remain intact at the crush site serving as direct pathways along which the axons travel to re-innervate their former end organs (Gutmann, Guttmann, Medawar & Young, 1942; Young, 1949). In these conditions return of function is rapid and to all intents and purposes complete. When a somatic nerve trunk is severed, on the other hand, and especially when a large gap remains between the cut ends, some delay and criss-crossing of axons occurs and many regenerate along sheaths leading to end-organs differing from those with which they were originally connected. The degree of impairment of function largely depends on the proportion of nerve fibres making wrong connexions.

Few studies have been made on the process of regeneration in the non-medullated nerve fibres in the autonomic nervous system. This is partly due to the greater difficulty in staining these fine fibres and to the comparative lack of precision with which return of function can be measured in most of the viscera innervated by autonomic nerves.

Several features in the structure of non-medullated nerve trunks suggest the possibility that the process of regeneration in these may differ appreciably from that in somatic nerves containing medullated fibres. There are marked differences between the sheaths of the two varieties of fibres.

The sheath of a medullated axon consists of three distinct components covering the outer surface of the myelin. Immediately around the myelin is a layer of protoplasm of a satellite cell, the cell of Schwann. This is fully enclosed in a tube of more rigid material whose inner wall is the neurilemma, a thin, smooth membrane, composed of very fine fibres. Outside the neurilemma the wall of the tube is further strengthened by coarser collagen fibres and continued as the endoneurium, by which various fibres are bound together. Each medullated fibre has an individual sheath and each sheath contains a single axon. In contrast, in non-medullated fibres, the sheath forms a continuous network composed of fine trabeculae of varying length and breadth (Nageotte, 1932; Gasser, 1952). The protoplasm of the Schwann cells contained within the trabeculae thus forms a syncytium. Around the trabeculae is



a thin but resistant membrane which corresponds to the neurilemma. The network is enclosed in endoneurium. Each branch of the network is a composite fibre containing a variable number, often large, of axons in the protoplasm. The protoplasm enclosed in the sheath is divided into septa of uniform thickness which separate axon from axon.

In a nerve trunk containing both medullated and non-medullated fibres, the latter come into close association with the sheaths of the former. The medullated fibres frequently pass through the loops formed in the network of the non-medullated fibre sheaths (Nageotte, 1932).

From the above description it might be expected that there is less precise direction of regenerating axons in the diffuse sheath of a non-medullated nerve trunk compared with the situation in a medullated nerve where the axons are guided along individual tubes.

Another striking difference between medullated and non-medullated fibres is seen in the behaviour of their respective Schwann cells following interruption of the axons. In medullated nerves these cells rapidly begin to proliferate, not only close to the lesion but throughout the length of the peripheral stump. Furthermore, in the region of a lesion in medullated fibres, the Schwann cells migrate and elongate into strands, which bridge the gap between the two cut ends (Ingebrigtsen, 1916; Abercrombie & Johnson, 1942). This outgrowth is of great importance in providing conducting pathways along which axons from the central stump reach the peripheral tubes (Young, 1942). The Schwann cell nuclei of non-medullated fibres, in contrast, show little or no proliferation as a result of degeneration of the axons (Tuckett, 1896; Joseph, 1947). As far as can be ascertained, however, no attempts have been made to determine whether the migratory property also is absent in non-medullated nerves.

In the present investigation regeneration in non-medullated fibres of the vagus of the rabbit has been studied. The vagus was selected because of the long lengths over which regeneration can be observed, enabling lesions to be made at different levels in the nerve trunk. The rabbit proved the most convenient species for three reasons. In the first place the vagus nerves at the level of the diaphragm in this animal are fairly constant in arrangement and position, being usually in the form of two discrete nerve trunks in contrast to the nerve plexuses found in the cat and dog. Secondly, the abdominal vagus nerves of the rabbit are composed almost entirely of non-medullated fibres; other species examined contained a larger proportion of medullated fibres. Thirdly, electrical stimulation of the rabbit vagus regularly produces a sharp increase in intragastric pressure, which thus serves as a suitable index of return of function. Similar stimulation in the cat and dog results in more variable effects.

#### METHODS

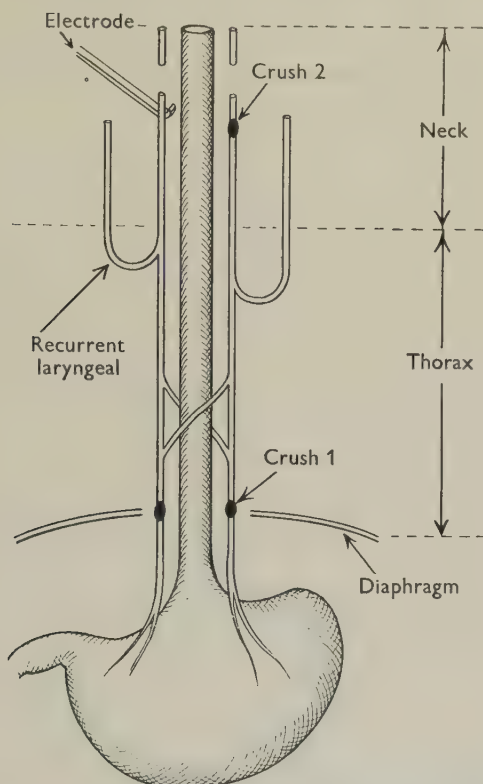
Adult rabbits of various breeds were employed throughout this investigation.

#### *Lesions of the vagus nerve*

A crush lesion of the vagus nerve was employed as a means of studying regeneration. The nerve was compressed for 10 sec. with a pair of watchmaker's forceps having smooth blades of 1 mm. width. Study of sections of medullated nerves fixed



at various times after such a lesion has shown that axons are interrupted, but the connective tissue sheaths are left intact and the regenerating fibres can advance along their original tubes to regain their old connexions (Gutmann *et al.* 1942; Young, 1949). Crushing a medullated nerve in this way therefore provides a valuable means of producing a standard lesion from which recovery is rapid and complete.



Text-fig. 1. Diagram showing the levels of the lesions: crush 1, at the level of the diaphragm; crush 2, at the level of the lower border of the thyroid cartilage.

The rabbits were anaesthetized with intravenous pentobarbitone sodium (Nembutal) supplemented with ether. Full aseptic precautions were observed. The lesions were standardized as follows:

(a) *At the level of the diaphragm* (Text-fig. 1). In a series of rabbits the anterior and posterior abdominal vagi were crushed, great care being taken that any additional branches were dealt with. In another series 15–20 mm. of the anterior and posterior nerves were resected.

(b) *At the level of thyroid cartilage* (Text-fig. 1). In a third group either the right or left cervical vagus was crushed opposite the lower border of the thyroid cartilage.

*Return of function*

At varying intervals after the lesions, the animals were biopsied. Return of function of the vagus nerves was tested and specimens of the nerves removed for histological examination. The methods varied depending on whether the lesions of the nerves were at the level of the diaphragm or in the neck.

(a) *After lesions at the level of the diaphragm.* Return of function to the stomach was measured by observing the effect of intragastric pressure of stimulating electrically the right and left vagus nerves in the neck (Text-fig. 1). The animals were anaesthetized with intravenous pentobarbitone sodium and maintained under anaesthesia with ether.

The abdomen was opened by a long mid-line incision, and a gastrotomy opening was made in the greater curvature of the stomach. A balloon, connected by pressure rubber tubing to a Marey's tambour recorder, was introduced into the lumen and the opening in the stomach closed by means of a purse string suture firmly tied around the rubber tube. The system was filled with air and intragastric pressure changes recorded on a revolving drum and calibrated by means of a water manometer. Pressure of from 8 to 12 cm. of water was employed and with the size of the balloon used about 25 ml. of air was required to produce such pressures. In all cases this volume was below that required to take up all the 'slack' in the balloon. The abdomen was widely opened, the abdominal walls being retracted in the manner suggested by Sollmann (1923) so that a trough was formed in which the stomach lay and the whole was kept moist with normal saline at 38° C. The right and left vagus nerves were demonstrated in the neck and divided at the level of the thyroid cartilage. The peripheral cut ends were stimulated in turn and the effect on intragastric pressure recorded. By stimulating only the peripheral cut ends, the complication of central stimulation with reflex effects on the viscera was avoided.

(b) *After lesions of the cervical vagus nerves.* The right and left vagus nerves were divided in the neck above the level of the crush and the effect of stimulation of the peripheral cut ends recorded (Text-fig. 1). The normal vagus was stimulated first and this acted as a convenient control. The treated vagus was stimulated proximal to the site of crush. The following observations were made:

(1) Effect on intragastric pressure was recorded by the method described above.

(2) Effect on the vocal cords. An incision through the thyrohyoid membrane allowed direct observations on the movement of the vocal cords. Re-innervation of the laryngeal muscles was assumed when stimulation of the treated vagus produced detectable movement of the vocal cord.

(3) Effect on the heart rate and blood pressure was recorded from the carotid artery.

The nerves were stimulated by rectangular voltage pulses delivered to the nerves through 30 s.w.g. platinum wire electrodes insulated with 'Perspex' from the surrounding tissues. The parameters of stimulation were varied experimentally over a wide range (pulse length 0.1–100 msec., pulse voltage 1–25 V., repetition rate 2–200 per sec.), and maximal responses from the stomach were obtained, in practice, by pulses of 0.1, 1.0 or 10 msec. duration at 10–25 V. amplitude at a repetition rate of 40 per sec. This applied in both normal and regenerating nerves.

## HISTOLOGICAL METHODS

The technique used for staining all axons in both medullated and non-medullated was the pyridine-silver method described by Ranson & Davenport (1931). The medullated fibres were demonstrated by the Weigert method which stains the myelin sheaths black. Details of the above methods and of counting and measuring fibres are described by Evans & Murray (1954).

Estimates of the total number of axons present in pyridine-silver preparations were made by a sampling method, the detail being as follows: the image of the cross-section of a nerve was projected at a magnification of 500 on paper of uniform thickness and the outline traced. This area was cut out and weighed and the cross-sectional area of the nerve calculated. The counts were made with a binocular microscope ( $\times 12$  ocular,  $\times 100$  objective) at a magnification of 1200 diameters. A 1 mm.<sup>2</sup> graticule was placed in one eyepiece and the area of nerve covered by each square calculated. A selection of squares was made from a table of random numbers and the numbers of axons in each square counted. The total area counted was between 17 and 33% of the total cross-section of each nerve. In the abdominal vagus nerve, where the axons are evenly distributed throughout the section, statistical analysis showed that counting of 17% of the total area gave a sufficiently consistent and therefore an adequate estimate of the total count. In the case of the regenerating recurrent laryngeal nerve the fibre population was unevenly distributed. The segment containing the large medullated fibres was sampled separately from that containing small fibres. Owing to the unevenness of fibre distribution in both segments as much as 33% of the total area of each segment was counted.

A special note is required in the case of animals subjected to division of the vagus with excision of a length at the level of the diaphragm. In these, it was necessary to remove the lower oesophagus, upper stomach and the nerves in one block for an examination of the neuroma and the orientation of the regenerating axons in relation to the distal nerve stumps. The complete specimen was lightly stretched on a glass frame and put in Bouin fixative (sat. aq. picric acid 75 ml.; 40% formaldehyde 25 ml.; glacial acetic acid 5 ml.). The specimen was subsequently embedded in paraffin, cut transversely at 10  $\mu$  thickness, and stained by the Bodian method for axons.

## RESULTS

For a clear understanding of the results, it is convenient to consider first the fibre content of the normal vagus nerve in the rabbit, then the process of regeneration as measured by return of function and finally the histological findings in regenerating nerves and to correlate these with the functional results.

(1) *Histological appearances of the normal vagus nerve*

An analysis of the fibre composition of the vagus and its branches in the rabbit has been made by Evans & Murray (1954) and the findings relevant to the present study are summarized in Table 1. It will be seen that the cervical vagus is composed of both medullated and non-medullated fibres, whilst the abdominal vagus consists almost entirely of non-medullated and the recurrent laryngeal of medullated fibres (Pl. 1, fig. 1; Pl. 2, figs. 7, 8).



Furthermore, we found that the great majority of the non-medullated fibres of the abdominal vagus nerves are afferent, only about one-tenth of the total being motor to the abdominal viscera. Observations were also made on the number of 'adventitial fibres' present in the abdominal vagus trunks. In three rabbits, 1372, 1721 and 569 axons were present (Pl. 1, fig. 5), and these were presumed to have been added to the vagus nerves in the thorax from communications with the sympathetic trunks.

Table 1. *Number of medullated and non-medullated fibres in the normal vagus and recurrent laryngeal nerves of the rabbit*

(The table shows the mean and standard deviation from the mean and, in brackets, the number of specimens counted.)

Specimens	Medullated fibres	Total number of axons	Non-medullated axons
Cervical vagus	2914 $\pm$ 207 (5)	23,132 $\pm$ 980 (6)	20,000 (approx.)*
Recurrent laryngeal			
(1) Laryngeal bundle	277 $\pm$ 14 (6)	—	(1) } 62 $\pm$ 9 (6)
(2) Tracheal and oesophageal bundle	400–600†	—	(2) }
Abdominal vagus nerves	65 $\pm$ 4 (4)	26,178 $\pm$ 1315 (6)	26,000 (approx.)*

\* In counts of Ranson preparations of cervical and abdominal vagi no attempt was made to differentiate medullated and non-medullated fibres. The approximate number of non-medullated axons was determined by subtracting the number of medullated axons from the total number of axons.

† No mean value could be determined as the number of fibres present in the individual specimens counted was very variable.

## (2) *Functional results*

### (a) *Normal response of the stomach to stimulation of the vagus nerve*

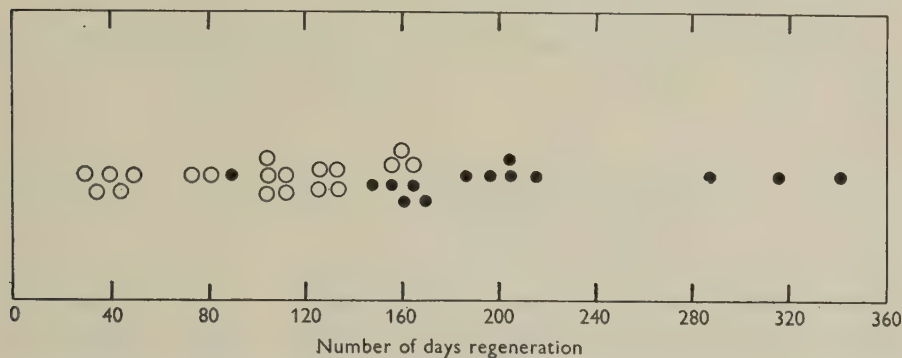
In a series of thirty-six normal rabbits, we obtained unequivocal results on stimulating the peripheral cut ends of the right and left vagus nerves in the neck. In all cases a sharp rise in intragastric pressure was produced, of the order of 15 cm. of water (Text-fig. 5A). The latent period was always brief, in all cases less than 2 sec. In spite of attempts to vary tonus and activity of the stomach by feeding the rabbit within an hour or two before biopsy or withholding solid food for as long as 72 hr., or varying the pressure in the balloon, the response was always similar in character. Stimulation of the right and left vagus nerves in the neck in the majority of cases gave a similar degree of response, but in a few cases the left had a slightly greater effect than the right. The reverse was never observed. This confirms the findings of M'Crea, M'Swiney & Stopford (1925), and emphasizes that the effects on the rabbit's stomach do not depend to the same extent on the degree of tone and peristaltic activity as in the cat and dog.

In all thirty-six control animals, the rise in intragastric pressure on stimulation of the vagus nerves in the neck was abolished by crushing or cutting the nerves at the level of the diaphragm.

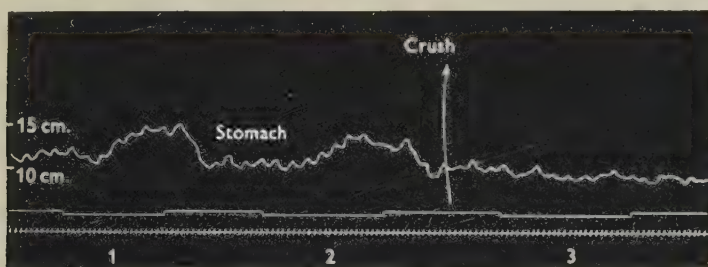
### (b) *Return of function after crushing the vagus nerves at the level of the diaphragm*

At varying intervals after crushing the vagus nerves at the level of the diaphragm, the animals were brought to biopsy. The results are summarized in Text-fig. 2. Up to 140 days after the lesions, the animals, with one exception (90 days), showed no

rise in intragastric pressure. From 140 to 170 days there was a transition period when the results were mixed, only a proportion showing a rise in intragastric pressure. With post-operative survival periods greater than 170 days, function had returned in all the animals tested. That the rise in intragastric pressure was due to regeneration of the abdominal vagus nerves was shown by the fact that in all cases it was abolished by crushing or cutting the nerves distal to the original crushes



Text-fig. 2. Regeneration following crushing at the level of the diaphragm. ●, rise of intragastric pressure on electrical stimulation; ○, no rise of intragastric pressure on electrical stimulation.



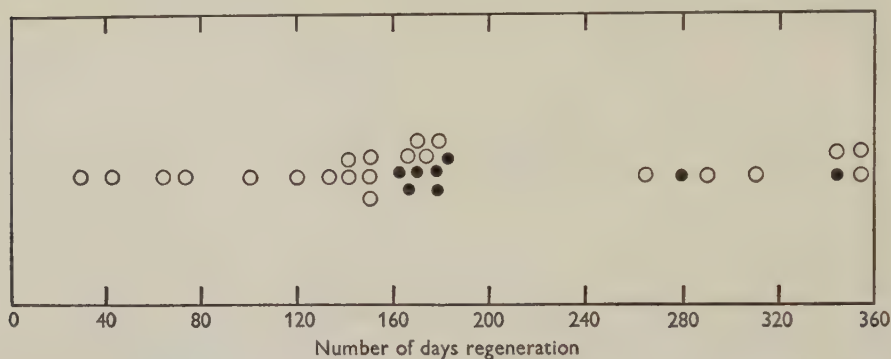
Text-fig. 3. (Rabbit 4.) Intragastric pressure recording showing a positive response after a lapse of 150 days following crushes at the level of the diaphragm. The response was less in amplitude and showed a considerable delay compared with the normal (Text-fig. 5 A). The rise in intragastric pressure was abolished by crushing the nerves distal to the original lesions. Time intervals on the tracings equal 1 sec.

(Text-fig. 3). The intragastric pressure response obtained in the early regenerating period (140–220 days) was subnormal in amplitude and showed a prolonged latent period of about 5 sec. However, in the three animals with post-operative survival periods of 285, 315 and 350 days the latent period and amplitude of the response approached normal. Thus we see that there is a period of increasing perfection of function extending over about 100 days.

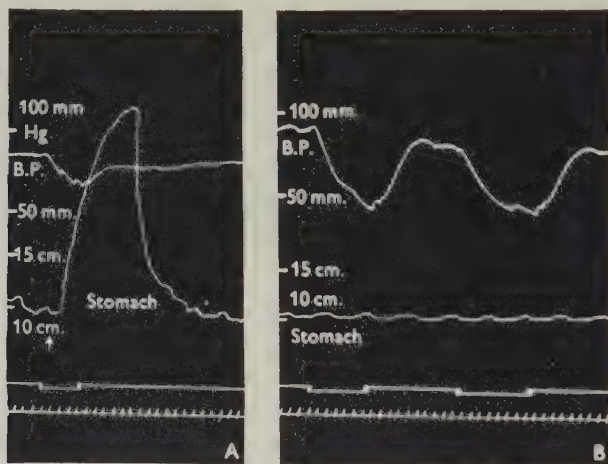
This fact suggests that stimulation of one point in the myenteric plexus by a regenerated vagus fibre is not transmitted by the plexus over a large area of the stomach wall. Presumably the myenteric plexus consists of a series of units, each having an individual vagal nerve supply (White, Smithwick & Simeone, 1952).

(c) *Return of function after division of the vagus nerves at the level of the diaphragm*

Text-fig. 4 summarizes the findings obtained in the series in which 15–20 mm. of the abdominal vagus nerves had been resected. As might be expected the return of function is not as sharply defined as in the series of crushed nerves. In each of the eight animals giving a positive response, the increase in intragastric pressure was abolished by cutting the nerves distal to the gaps.



Text-fig. 4. Regeneration following division at the level of the diaphragm. ●, rise of intragastric pressure on electrical stimulation; ○, no rise of intragastric pressure on electrical stimulation.



Text-fig. 5. (Rabbit 19.) Intragastric and blood-pressure records from an animal in which the left cervical vagus had been crushed 670 days previously. A, right side showing normal responses; B, left side showing a return of function to the heart but none to the stomach. Time intervals on the tracings equal 1 sec.

In all cases when function had returned, regeneration was only partial as indicated by a long latent period and a small rise in intragastric pressure. It was observed that after a crush there was a period of increasing perfection of function, but after division this was not the case, at least up to 350 days.



(d) *Return of function after crush of the right or left vagus in the neck*

We were unable to demonstrate return of function to the stomach in any of the animals subjected to a crush of the cervical vagus (Text-fig. 5). This was a striking phenomenon and the cause became apparent only after histological investigation.

Table 2. *Regeneration following crushing in the neck at level of thyroid cartilage*

(0=no reaction on electrical stimulation; +=reaction on electrical stimulation.)

Rabbit number	Right or left vagus	Number of days regeneration	Stomach	Heart	Vocal cords
91	R	42	0	0	0
92	L	51	0	0	0
72	L	61	0	0	0
158	R	70	0	+ slight	+
1	L	75	0	Not tested	+
38	R	84	0	0	+
53	L	110	0	+	+
18	L	135	0	+	Not tested
9	R	164	0	+	+
34	R	168	0	+	+
8	L	183	0	+	+
10	R	187	0	+	+
46	R	200	0	Not tested	Not tested
16	L	205	0	0	+
28	L	307	0	+	+
11	R	311	0	+	+
12	L	436	0	Not tested	Not tested
7	R	670	0	+ slight	+
19	L	670	0	+	+

On the other hand, the return of function to the larynx and heart presented a much more uniform picture. The results are summarized in Table 2. The distances as measured in four animals from the site of crush to the three structures in which function was tested is shown in Table 3.

Table 3. *Distances measured along the nerves from site of the crushes in the neck to the larynx, heart and diaphragm*

(The measurements were made in four rabbits.)

Rabbit number	Weight (kg.)	Larynx		Heart* (cm.)	Diaphragm (cm.)	Stomach† (cm.)
		Right (cm.)	Left (cm.)			
112	2.1	13.5	17	11.5	16.5	8.5
196	2.7	15	18	11	18	10
195	3.0	15	18	11.5	17	10.5
10	3.3	12.5	16	10.5	17.5	10.5

\* Measurement to the level of the atria.

† Distance from the level of the diaphragm to the pyloro-duodenal junction as measured along a line mid-way between the greater and lesser curvatures.

(3) *Histological results*(a) *Nerve crush at the level of the diaphragm*

Twenty days after the crush, there were 2709 axons present 20 mm. distal to the crush (Table 4). This indicates a rate of growth of axon tips greater than 1 mm. per day. It was apparent that the regenerating axons were smaller than normal in

diameter (Pl. 1, fig. 2). After a lapse of 40 days following a crush, the axons at a level 20 mm. distally were more numerous than at 20 days, but still appeared smaller than normal. By 70 days the fibre density 20 mm. distal to the crush had returned to normal (Table 4) and the axons appeared to be normal in size.

Table 4. *Non-medullated fibre counts 10 mm. proximal and 20 mm. distal to a crush of the anterior vagus nerve at the level of the diaphragm*

Rabbit number	Regeneration period in days	Number of axons	
		Proximal	Distal
131	20	8,943	2,709
163	50	18,633	10,518
150	70	7,564	7,770

Before function is restored, it may be presumed that the following processes must occur: (a) the regenerating axons must grow across the lesion; (b) these axons must grow down to ganglion cells in the myenteric plexus; (c) synaptic connexions must be re-established; (d) increase in diameter of the regenerating axons may have to occur. Our histological investigations suggest that growth across the crush lesion and rate of growth of axon tips cannot be mainly responsible for the delay. Very little is known about processes (c) and (d) in non-medullated fibres. We consider that another factor may contribute to the delay in this situation. Owing to the syncytial nature of the nerve sheaths, the regenerating non-medullated fibres have no specific guiding channels along which to reach appropriate end-organs. It is likely, therefore, that many axons fail or take a longer time than anticipated, to make functional connexions. This effect would be accentuated by the dispersed nature of the ganglion cells in the myenteric plexus. Further, the lack of guiding tubes and the widely scattered end-organs may well explain the relatively long period of increasing perfection of function.

*(b) Section with excision of 15–20 mm. of nerve at the level of the diaphragm*

From transverse and longitudinal sections a picture could be built up of the axons growing out from the proximal cut end. The axons grew out in all directions, some passing cranially along the outside of the nerve trunks and a variable number successfully bridging the gaps and growing into the distal nerve trunks (Pl. 1, fig. 6). In some, large numbers of axons grew into the oesophageal wall. Of the thirty cases which were investigated for return of function, four were rejected because of inadequate staining and three were used for longitudinal section. Of the remaining twenty-three specimens, all showed the presence of axons in the distal nerve stump. In some there were relatively large numbers of axons and in others few. We were unable to observe any correlation between the number of axons present in the distal stumps and the return of function. In the later stages of regeneration, i.e. 260 days and more after the division, there appeared to be no greater number of axons than at the earlier periods. Axons were present in the distal stumps as early as 42 days after the division. A constant and striking finding in transverse sections of the distal stumps was that the axons which successfully bridged the gap were arranged around the periphery of the section, the more central portions being almost devoid of axons.

It is interesting to note that in rabbits with regenerating periods greater than 160 days, only eight of the eighteen animals showed a return of function, although in all cases axons had successfully regenerated down the distal nerve trunks. Several factors could contribute to this state of affairs.

(1) Although in all cases some axons are successful in bridging the gap, in some the proportion is relatively small compared with the normal number.

(2) As we have shown (Evans & Murray, 1954) a large proportion of the non-medullated fibres in the abdominal vagus nerves are afferent, and thus the number of successful efferent fibres may be very small.

(3) It appears that non-medullated fibres do not have specific tubes to guide them back directly to an end organ and thus the likelihood of functional connexion being established is diminished.

*(c) Nerve crush at the level of the thyroid cartilage*

After a crush in the neck the medullated fibres destined for the recurrent laryngeal nerve behaved as anticipated. Function returned rapidly, e.g. in 75 days over a distance of 17 cm. (rabbit 1) and there were 220 medullated fibres present in the recurrent laryngeal nerve adjacent to the larynx.

Table 5. *Non-medullated fibre counts in regenerating recurrent laryngeal and abdominal vagus nerves after a crush of the left cervical vagus*

Rabbit number	Regeneration period in days	Recurrent laryngeal	Abdominal vagus nerves
126	280	9,443	2,485
54	550	10,649	5,805
19	670	7,243	2,466

On the other hand, the outstanding finding after crushing the vagus in the neck was that function failed to return to the stomach even after a lapse of 670 days. Histological examination of the abdominal vagus nerves at the level of the diaphragm showed that large segments of the section were almost entirely devoid of axons, even after very prolonged regeneration times (Pl. 1, fig. 4). The appearances were similar to those found in the abdominal vagus nerves 3 weeks after cutting one vagus in the neck (Evans & Murray, 1954). It was thus clear that few axons had succeeded in reaching the abdomen even after very prolonged regeneration times. In order to study this finding more fully, quantitative estimations of the number of regenerating axons reaching the abdomen were made in the following manner: in three of the rabbits with long-term cervical crush, the contralateral cervical vagus was cut and a segment removed. After allowing a period of 3 weeks for fibres to degenerate, the animals were biopsied and the abdominal vagus nerves removed for pyridine-silver staining. The nerves contained remarkably few fibres which were distributed throughout the section (Pl. 1, fig. 3). Table 5 shows the fibre content of the nerves in these three instances. The numbers found in the present series are slightly larger than those of the 'adventitial fibres' discussed previously (Pl. 1, fig. 5). This small increase in number is presumably due to a few vagal fibres having grown successfully from the level in the neck to the abdominal vagus nerves. Some of these



fibres may have been motor, but if so, they were evidently too few to produce contraction of the stomach when stimulated.

*'Guidance' of the growing tips of non-medullated fibres by medullated fibre sheaths* (Evans & Murray, 1953). The fate of the regenerating non-medullated fibres was elucidated when the recurrent laryngeal, cardiac and bronchial branches of the vagus were examined. These presented a remarkable appearance in that groups of non-medullated fibres were observed forming crescents or rings surrounding the medullated fibres in these branches. This was particularly striking in the recurrent laryngeal nerve, which normally contains very few non-medullated fibres (Pl. 2, fig. 8). In the regenerating nerve, on the other hand, all the medullated fibres were surrounded by non-medullated axons (Pl. 2, fig. 9); in some cases as many as twenty-five to thirty of these were found related to a single medullated fibre (Pl. 2, fig. 10a). This appearance was observed in recurrent laryngeal nerves both after long and short regeneration periods. The diverted axons regenerated along the whole length of the nerve, a distance of 14–17 cm. (Table 3) from the site of the crush and entered the laryngeal muscles. Bielschowsky-Gros preparations of these muscles showed numerous fine axons growing alongside the muscle fibres, but making no apparent connexion with the end plates.

Counts of the total number of non-medullated fibres present in the regenerating recurrent laryngeal nerve at the level of the clavicle were made in three rabbits with long survival periods following a cervical vagus crush. Table 5 shows the values obtained and indicates that the number of non-medullated fibres which have been diverted into the recurrent laryngeal nerve accounted for almost half of the total number of non-medullated fibres normally present in the cervical vagus. This is based on the assumption that each regenerating non-medullated axon produced only one sprout. Additional groups of non-medullated fibres were found surrounding the medullated fibres in the cardiac and bronchial branches and these undoubtedly accounted for the majority of the remaining regenerating non-medullated fibres.

*Position of the non-medullated axons growing along the sheaths of the medullated fibres.* It was not possible to decide the position of the non-medullated axons in relation to the various components of the sheath of the medullated fibres simply from observations made on sections of regenerating nerve stained by the pyridine-silver method. Several methods of counter-staining were tried, the best results being obtained with that described by Foley (1938). Unfortunately, however, it was not possible to distinguish the boundaries between the Schwann cells and the neurilemma and between the neurilemma and the endoneurium (Pl. 2, fig. 11).

More definite, but still indirect, evidence regarding the position of non-medullated axons in the regenerating recurrent laryngeal nerve was obtained when the large medullated fibres in this nerve were caused to degenerate. A two-stage operation was carried out on two rabbits. The first stage consisted of a crush of the vagus trunk in the neck, after which regeneration was permitted in the two animals for 240 and 260 days respectively. Then extracranial section of the nerve between the skull and the upper pole of the nodose ganglion was performed and the animals allowed to survive for a further period of 30 days. In the rabbit such extracranial section of the vagus results in degeneration of all the large medullated fibres present in the laryngeal bundle of the recurrent laryngeal nerve (Evans & Murray, 1954).

These fibres, which constitute the motor innervation of the laryngeal muscles, have their cells of origin in the medulla. On the other hand, the majority (about 75%) of the non-medullated fibres present in the cervical vagus have their cells of origin in the nodose ganglion and these therefore survive supranodose section of the nerve (Evans & Murray, 1954). Examination of pyridine-silver stained sections of the laryngeal bundle of the recurrent laryngeal nerve in these two animals showed the presence of non-medullated fibres in large numbers. This demonstrates that they are not sprouts of regenerating medullated fibres but are, in fact, regenerating non-medullated fibres which have been diverted into the recurrent laryngeal nerve. Most of the fibres were situated within the sheaths of the now degenerated medullated fibres, occupying the centre of the tubes (Pl. 2, fig. 10*c*) in contrast to the more peripheral position they occupy in the presence of medullated fibres. This suggests that these axons were originally placed between the neurilemmal membrane and the outer surface of the protoplasmic layer composed of Schwann cells. When the medullated fibre degenerates, the Schwann cells proliferate and come to occupy the centre of the tube. Evidently most of the non-medullated axons adhere to the surface of the Schwann cells and thus become reorientated.

In the early period following a crush of the cervical vagus the growing tips of the non-medullated axons must presumably penetrate the endoneurium and neurilemma to reach the centre of the tube of the medullated fibre. It was first considered that this penetration would be most likely to occur at the site of the crush. However, this was not the case as in sections taken immediately distal to the crush the non-medullated axons were not orientated in rings around the medullated fibres. This appearance was visible only in sections taken at several millimetres below the crush, and it was evident that more and more axons became added to the rings as the nerve was traced distally.

In the early regenerating period (20 days and less) the outgrowths of the non-medullated axons that have thus penetrated the sheaths grow amongst the thin sprouts of the regenerating medullated fibres. At this stage both types of axons were found distributed amongst the proliferating Schwann cells occupying the centres of the tubes and no clear distinction between the two varieties was possible. Very soon, however, one or more of the thin outgrowths (Pl. 2, fig. 12) within each tube thickens and becomes medullated. The larger fibres originate as outgrowths from a medullated parent fibre and the thickening process begins before the tips of the fibres have made contact with end organs. The growth in diameter which these fibres undergo causes the remaining axons in the tube to be displaced to a more peripheral position (Pl. 2, figs. 10*a, b*). A similar displacement occurs during regeneration of a purely medullated somatic nerve. At an early stage following a crush each peripheral tube becomes populated by a number of fine fibres all derived from the one central axon. Later, when one of these fibres reaches a suitable end-organ, it begins to increase in diameter, displacing the remaining axons towards the periphery of the tube and ultimately causing them to disappear (Sanders & Young, 1944; Aitken, Sharman & Young, 1947). From these results it may be seen that, apart from the principal axon, all sprouts arising from the medullated parent fibre are firstly displaced to the periphery of the tube and finally the majority disappear; the non-medullated axons regenerating down such tubes, although similarly displaced, persist, even after full maturation of the medullated axon.

## DISCUSSION

The results of this study confirm that the central stumps of non-medullated axons which have been interrupted by crushing or cutting a nerve trunk have a strong regenerative capacity. When the vagus nerve is crushed the axons of non-medullated as well as those of medullated fibres grow directly through the crushed region to invade the distal stump. The subsequent behaviour of the non-medullated axons is profoundly influenced by the presence or absence of medullated fibres. When the abdominal vagus nerve, which consists almost entirely of non-medullated fibres, was crushed the regenerating axons in the peripheral stumps assumed a distribution pattern indistinguishable from the normal. Ultimately they reached end-organs in the stomach wall and function became re-established. Below a lesion of the cervical vagus, on the other hand most of the non-medullated axons became arranged alongside the sheaths of the medullated fibres and were thus diverted from their old pathways. In consequence no functional re-innervation of the stomach occurred.

The part played by the sheaths of medullated fibres in directing the path taken by new axons during regeneration has been studied in detail by Holmes & Young (1942). During regeneration of a nerve composed of myelinated fibres the pattern of the peripheral stump of the nerve is maintained by the neurilemma and endoneurium. These structures persist as a continuous tube leading to the end-organs. The Schwann cells proliferate rapidly soon after the interruption of the axons and they come to occupy the centre of the tube. The nuclei and the protoplasm of the Schwann cells become greatly elongated. During the early stages of regeneration Holmes & Young found that the new axons grew along the interface between the inner wall of the neurilemmal sheath and the surface of the Schwann cells. When a medullated nerve is crushed by the method used in the present study the great majority of the connective tissue sheaths of the medullated fibres are not interrupted and thus most of the regenerating axons grow along their original tubes.

Evidently the sheaths of non-medullated fibres do not provide such efficient pathways for the regenerating axons. This might be expected from the account of the structure of such sheaths given by Nageotte (1932) and Gasser (1952). The syncytial arrangement of the Schwann sheath which they described would provide ample opportunity for regenerating non-medullated axons to wander about within the nerve trunk. Nageotte found that medullated fibres frequently traversed the interstices of this syncytium. Non-medullated axons regenerating along the syncytium thus come in close contact with the endoneural sheaths of medullated fibres, and they grow alongside these fibres in preference to their old pathways.

The experimental findings described above demonstrate marked differences in the process of regeneration of medullated and non-medullated fibres.

## SUMMARY

An experimental study has been made of regeneration of non-medullated fibres in the vagus nerve of the rabbit. The vagus nerve in the neck is composed of medullated and non-medullated fibres whereas in the abdomen, the nerves are almost entirely non-medullated. Thus the behaviour of regenerating non-medullated fibres in the presence and absence of medullated fibres can be studied by lesions at these two levels.



1. Following a crush of the abdominal vagi, function returns uniformly to the stomach but is delayed. The delay is possibly due to the lack of specific guiding channels in non-medullated nerves.

2. Following a crush of the cervical vagus, no evidence of functional return to the stomach was found even after survival periods of 600 days. Histological findings showed that few non-medullated axons had succeeded in regenerating to the abdomen.

3. The fate of the regenerating non-medullated fibres was elucidated on examination of the recurrent laryngeal nerve. They had been diverted from their original pathway and 'guided' along the medullated fibres in the recurrent laryngeal nerve.

4. The majority of the regenerating non-medullated fibres were situated within the Schwann tubes of the medullated fibres in the recurrent laryngeal nerve.

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#### EXPLANATION OF PLATES

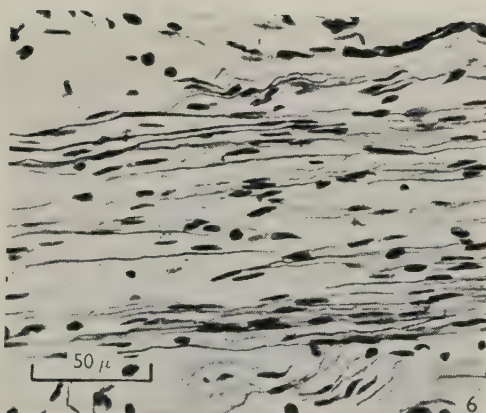
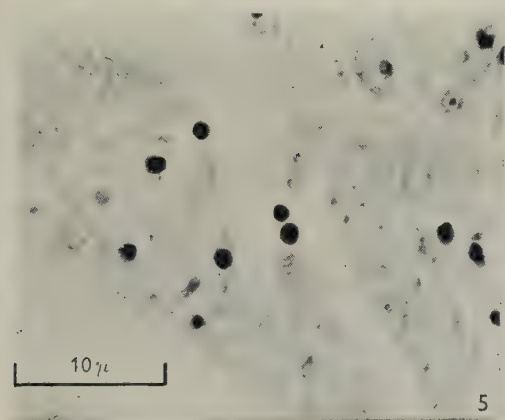
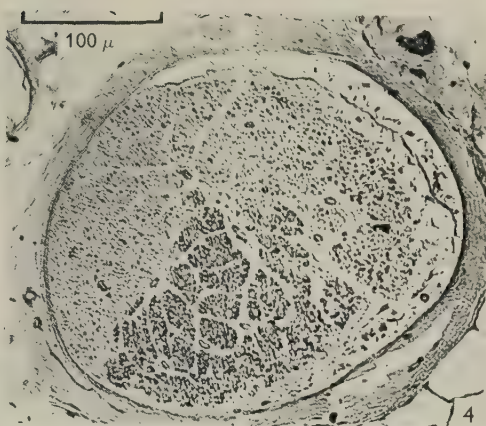
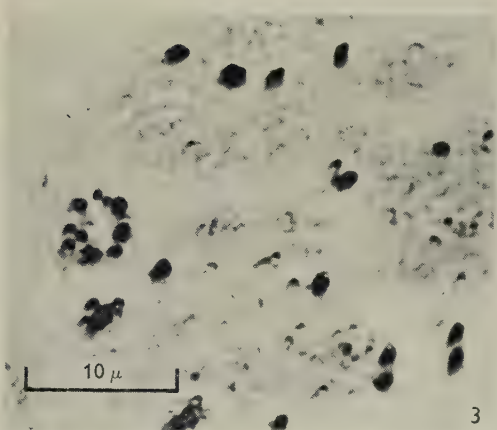
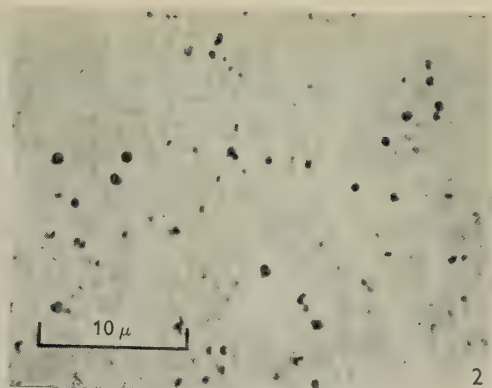
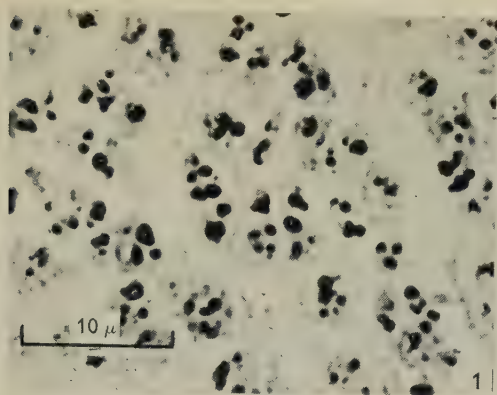
With the exception of Pl. 1, fig. 6, all are transverse sections stained by the pyridine-silver method.

##### PLATE 1

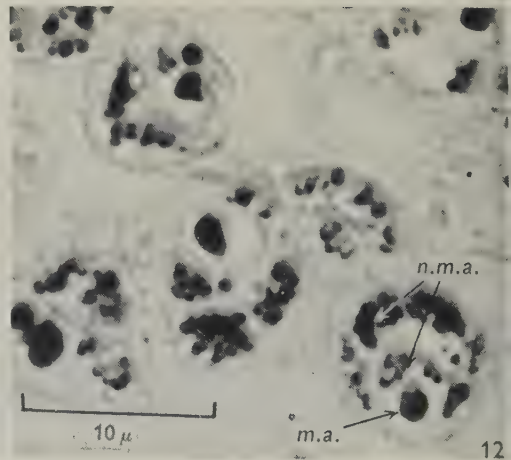
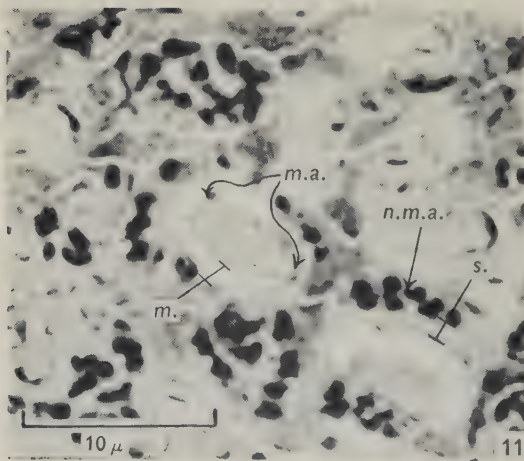
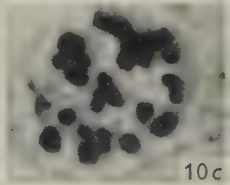
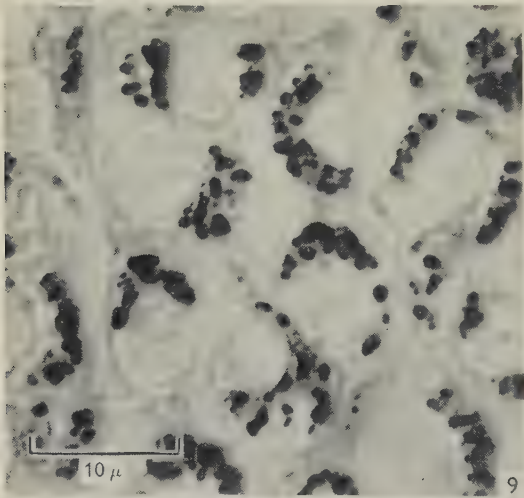
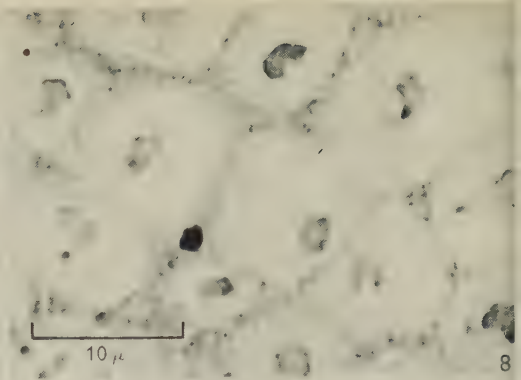
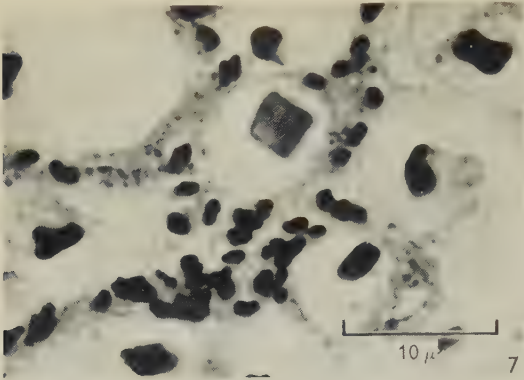
- Fig. 1. A segment of a normal posterior abdominal vagus with a high population of non-medullated axons.
- Fig. 2. Section of an anterior abdominal vagus trunk 20 mm. distal to a crush. Regeneration period of 20 days. The axons are few and uniformly distributed. They are smaller in diameter than normal.
- Fig. 3. A segment of the anterior abdominal vagus showing that few non-medullated fibres have grown back after a crush of the left cervical vagus 670 days previously. The right cervical vagus was divided 21 days before biopsy. On the left side, a group of regenerating non-medullated fibres are arranged around a medullated fibre tube.
- Fig. 4. Low-power view of the anterior abdominal vagus, 307 days after a left cervical crush. Few, if any, fibres have regenerated into the segment of nerve occupied by the left vagus (upper segment of section).
- Fig. 5. A segment of an anterior abdominal vagus nerve showing a few remaining axons after both cervical vagus nerves have been divided. The right nerve was cut 45 days and the left 21 days before the biopsy.
- Fig. 6. Longitudinal section of the lower part of a neuroma showing regenerating non-medullated fibres bridging a gap of 15 mm. to re-innervate the distal stump of the abdominal vagus nerve. A 15 mm. segment of the nerve at the level of the diaphragm had been removed 42 days before biopsy. Bodian stain.

##### PLATE 2

- Fig. 7. A segment of the normal left cervical vagus showing the medullated axons within their tubes and the non-medullated axons in the connective tissue between the tubes.
- Fig. 8. Section showing normal left recurrent laryngeal nerve with the medullated axons staining faintly within their tubes and one sharply stained non-medullated axon.
- Fig. 9. Section showing regenerating left recurrent laryngeal nerve 280 days after a crush of the left cervical vagus. Non-medullated axons are arranged around the tubes of the medullated fibres. Compare with the normal nerve (Pl. 2, fig. 8).
- Fig. 10. Showing orientation of non-medullated fibres in relation to the sheaths of medullated fibres in regenerating recurrent laryngeal nerves.
- Fig. 10a. 550 days after cervical crush. The large central axon is very faintly stained.
- Fig. 10b. 670 days after cervical crush showing the medullated axon darkly stained.
- Fig. 10c. 240 days regeneration after cervical crush followed by section of the vagus cranial to the nodose ganglion 30 days before biopsy. The medullated fibre has degenerated and the surviving non-medullated axons now occupy the centre of the tube.
- Fig. 11. Regenerating recurrent laryngeal nerve 280 days after a crush of the left cervical vagus showing the orientation of the non-medullated axons (*n.m.a.*) in relation to the medullated axon (*m.a.*). Most of these axons are separated from the myelin (*m.*) by a definite zone of the sheath (*s.*). One tube contains no medullated fibre and in this the non-medullated axons are scattered throughout the centre of the tube. Pyridine-silver, counter-stained by Foley's method.
- Fig. 12. Cervical vagus 2 cm. below a crush after 28 days' regeneration. At least one axon (*m.a.*) in each tube has already become medullated. Groups of non-medullated axons (*n.m.a.*) are arranged at the periphery and towards the centre of the tubes.







# THE SECONDARY OLFACTORY AREAS IN THE HUMAN BRAIN

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Neurologists have accepted for many years that olfactory sensation in the human subject are referred ultimately to the uncus, the anterior perforated substance, the septal areas in the subcallosal gyrus and possibly the hippocampal formation. This conclusion is based on the study of normal material, the comparison with Marchi experiments on lower animals and records of cases with lesions which had affected olfaction to a greater or lesser extent. None of these methods alone is entirely reliable, and hitherto it has not been possible to localize at all precisely the olfactory areas in the human brain.

During the past decade the mammalian olfactory connexions have been carefully studied by experimental methods. The observations which have been made unite to show that the distribution of fibres from the olfactory bulb is essentially similar in widely different groups of mammals, despite the marked displacement of the base of the telencephalon which is associated with the expansion of the cerebral cortex (Allison, 1953*a*). The main secondary olfactory areas have a characteristic arrangement of nerve cell-bodies and fibres. Hence they can usually be distinguished from the adjacent areas, and a satisfactory localization of the olfactory brain can be made on the basis of a study of normal material examined in the light of experimental findings.

This communication represents an attempt to apply the results of animal experimentation to the human brain. The areas in the human brain most probably receiving fibre accessions from the olfactory bulb, which may therefore be designated secondary olfactory areas, are described. The conclusions are based mainly upon a detailed comparison of the human forebrain with that of the monkey (*Macaca* and *Papio*) in which the olfactory connexions have been determined experimentally (Meyer & Allison, 1949). The pathological changes in a human brain following surgical transection of the olfactory tracts on one side provide confirmatory evidence for the conclusions given below.

The voluminous literature covering the morphology of this part of the human brain will not be reviewed here. References to earlier writers will be found in the works of Campbell (1905), Brodmann (1909), Cajal (1911), Johnston (1923), Rose (1927*a, b*, 1928, 1935), v. Economo (1927), Beck (1934) and Crosby & Humphrey (1941), whose descriptions form the basis of the present analysis.

## MATERIAL

Series of 20 $\mu$  transverse celloidin sections through two normal human brains were available, every twelfth section mounted and stained alternately for cell masses and myelinated fibres.

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The brain of a man aged 52, whose olfactory peduncle had been accidentally severed on one side 2 years before death during a prefrontal leucotomy, was made available for examination through the courtesy of Prof. Alfred Meyer of the Institute of Psychiatry, London. The brain had been fixed in formal-saline; every twenty-fifth section of a series of  $20\mu$  celloidin sections was mounted and stained with cresyl violet.

One normal human brain (man aged 49, dying of coronary thrombosis) was dissected and photographed; sections  $15\text{--}20\mu$  thick were cut in a transverse plane and stained by the Nissl, Weil and Bielschowski silver methods. From observations of these sections the position of the olfactory areas was marked on the photographs showing the surface of the brain (Pl. 1, figs. 1, 2). Photomicrographs of Nissl sections included in Pls. 1 and 2 show the disposition of most of the main basal olfactory areas.

#### OBSERVATIONS

*Anterior olfactory nucleus.* This nucleus is relatively small in man. In most mammals the nucleus forms a circle around the olfactory ventricle in the olfactory crus, but in man the olfactory crus is much attenuated and the olfactory ventricle obliterated, so that the nucleus is represented only as isolated masses of cells scattered throughout the length of the peduncular fibres. The dorsal part of the nucleus is, as Crosby & Humphrey (1941) state, conspicuous on account of its comparatively large pyramidal cells. The external part of the nucleus appears to be represented by small, discontinuous patches of small, deeply stained neurons, situated at the ventral border of the neocortex and continuing postero-laterally as far as the prepiriform area (it is included in the lateral part of the nucleus of Crosby & Humphrey). The external part of the nucleus had a fascicle of olfactory tract fibres closely associated with it. It is thus probable that in man, as in the monkey and lower mammals, only the dorsal and external parts of the nucleus receive olfactory fibres from the bulb. The other parts of the anterior olfactory nucleus (or area retrobulbaris of Rose) are comparatively small, and their position is described by Crosby & Humphrey.

*Olfactory tubercle.* The term 'anterior perforated substance' as applied to the human brain often refers to most of the area between the olfactory trigone and the optic chiasma, thus including structures other than the olfactory tubercle in the brains of lower mammals. The term 'olfactory tubercle' is employed here, as Rose and Crosby & Humphrey have used it, to designate the region on the basal surface of the frontal lobe between the olfactory tract (situated antero-laterally) and the nucleus of the diagonal band (which lies postero-medial to it).<sup>\*</sup> This has been subdivided by both Rose and Crosby & Humphrey, into three areas rostral (tol 1), middle (tol 2) and caudal (tol 3), although perhaps the terms 'antero-lateral', 'middle' and 'postero-medial' might be more appropriate to indicate their relative positions.

The general relationships of the olfactory tubercle in the human brain are the same as those in other mammals. On the surface of the brain the tubercle shows two distinct parts, one, adjacent to the olfactory tract, being smooth and rounded and

<sup>\*</sup> Many other definitions have been given. Thus Cajal's anterior perforated space includes the diagonal band and the Vogts' (1919) tuberculum olfactorium refers to the olfactory trigone alone (Rose's tol 1 and a small part of tol 2). The present usage is, however, more consistent with that given in the comparative descriptions.



the other perforated by many small blood vessels. In sections the characteristic lamination of the olfactory tubercle is most conspicuous in the antero-lateral part (tol 1 and tol 2): in this region a distinct band of small, closely packed pyramidal cells lies between the superficial plexiform layer and the deep layer of polymorphous cells. Further medially the layers are broken up, islets of Calleja are present (including the large medial islet of small, granular-type cells bordering on the septal areas) and the lamination is either very indistinct or absent altogether (mainly tol 3).

In myelin preparations, and even more clearly in silver preparations, a distinct fascicle of fibres can be traced from the medial side of the olfactory tract to the plexiform layer of the tubercle. These fibres in all probability end for the most part in tol 1, where they are shown on the surface of the brain in Pl. 1, fig. 2. They constitute the small bundle which has been termed 'medial olfactory stria' or 'medial olfacto-frontal fascicle'.

The anterior limb of the anterior commissure system, upon leaving the olfactory peduncle, penetrates the brain substance and comes to lie above the olfactory tubercle along with other small fascicles of fibres. Further posteriorly it joins the antero-ventral side of the relatively enormous mass of fibres belonging to the transverse and temporal limbs of the commissure.

*Septal areas.* These areas have been shown in the experiments on monkeys to receive very few if any, olfactory fibres directly. This is very probably true of the human brain also: certainly no significant contingent of fibres can be traced from the olfactory tract to the septal region.

*Prepiriform cortex.* This part of the allocortex has never been properly localized in the human brain. Many authors have described or figured parts of it, but even Cajal, Beck and Rose, who have all studied this region closely, failed to distinguish the olfactory from the non-olfactory cortex.

It has been observed in available material that the prepiriform cortex in the human brain, as in the monkey and lower mammals, has two parts. The frontal part is confined to a small, triangular area on the lateral side of the olfactory tract, and it is partly covered by the expanded neocortex of the medial orbital gyrus (so that in sections taken at some levels the olfactory tract fibres and prepiriform cortex appear to lie not on the surface of the brain but beneath a molecular layer of isocortex (Pl. 1, fig. 3). The frontal prepiriform cortex has a typical laminated structure, with a plexiform layer containing fibres from the olfactory tract, a band of fairly deeply stained pyramidal cells, and a polymorphous cell layer with ill-defined internal limits. This area is termed area prepiriformis 2 and 3 by Rose (1927*b*); part of the agranular insular cortex (parts of *ai* 4 and 5) by Rose (1928) and *Fk* and *Id* by v. Economo (1927).

Continuous at some levels (Pl. 2, fig. 5), with the small area of prepiriform cortex on the orbital surface of the frontal lobe is the much more extensive temporal prepiriform area. The olfactory tract fibres spread over it from the point of termination of the main bundle in the angle between frontal and temporal lobes. These fibres are expended in the plexiform layer, where many are not tangential (as in other cortical areas), but are turned inwards to end in relation to the dendrites of the pyramidal cells. In some regions this cortex presents a typical lamination

(which is very similar to that observed in the monkey or even in the rabbit), but towards the fringe of the area the conspicuous band of pyramidal cells is broken up and small islets of pyramidal cells occur. These are not so large as the very prominent islets in the main part of the entorhinal area, although the boundary between the two types of cortex is not always very distinct. If the disposition of the cells and the extent of the superficial layer of olfactory fibres (which is thicker than the tangential layer of fibres in other cortical areas) are taken together, however, the limits of the prepiriform cortex can be nearly determined (as shown in Pl. 1, fig. 2). Posteriorly, there is a narrow transition area between the prepiriform cortex and the cortical and medial amygdaloid nuclei, as in other animals; anteriorly and laterally the prepiriform cortex goes over into temporal and insular isocortex.

This primary olfactory cortex has been given various designations. Cajal included in his 'région olfactive centrale' in the human brain part of the entorhinal cortex. Most of the temporal prepiriform area as here defined falls into the entorhinal area of Rose (1927*b*)—mainly the anterior part of his *ey*; however, in a later study, Rose (1928) terms the same area agranular insular cortex—parts of *ai* 9 and *ai* 10. The temporal prepiriform area is included in v. Economo's area *Ha*. However, the extent of the temporal prepiriform area does correspond very nearly to that of the area temporalis insulae of Beck (1934), who investigated the myelo-architecture of the dorsal temporal region in detail. Beck ascribed the prepiriform cortex to a very small area on the surface of the temporal lobe immediately adjacent to the olfactory tract; he points out that the main characteristic of the area temporalis insulae is the prominence of the fibrous first layer, which becomes thicker in the posterior part of the area (that is to say, nearest the olfactory tract). Hence it must be concluded that in man, as in the monkey, Beck's area temporalis insulae is the prepiriform area and that the superficial fibrous layer is derived from the olfactory tract.

The temporal prepiriform area thus comprises a considerable part of the cortex of the gyrus ambiens; it is separated by the semi-annular sulcus from the amygdala and by a shallow depression from the main part of the entorhinal area in the hippocampal gyrus.

*Amygdaloid complex.* The amygdaloid complex in the human brain has been described by Johnston (1923), Hilpert (1928), Crosby & Humphrey (1941) and others. The terminology of Johnston and of Crosby & Humphrey, which is now standardized, has been employed in this description.

As already stated, most of the olfactory tract fibres turn forward to end in the temporal prepiriform cortex, but a relatively small number of these fibres can be seen passing back to the amygdala, where they apparently terminate in the superficial part of the cortico-medial group of nuclei, as in the monkey. Only the cortico-medial group of nuclei will be given consideration here, then; for a fuller account of the large and prominent baso-lateral nuclear masses reference may be made to the text of Crosby & Humphrey.

*Nucleus of the lateral olfactory tract.* This is represented only by two or three very small masses of deeply stained cells amongst the olfactory fibres between the edge of the medial nucleus and the cortical nucleus. It almost certainly receives olfactory terminals, but it is so much reduced in size that it can hardly be very significant functionally.

*Medial nucleus.* This nucleus, which lies near the entorhinal fissure, is relatively limited, and consists for the most part of small, rather faintly stained cells. As in the monkey, many olfactory fibres may be seen over the surface of the medial nucleus, and they apparently penetrate quite deeply into its antero-medial part.

*Cortical nucleus.* This is the largest of the cortico-medial nuclei. Olfactory fibres can be traced into the plexiform layer, where they probably terminate in relation to the dendrites of the neurons in about the anterior third of the nucleus. From analogy with the monkey it is unlikely that the tangential fibres over the more posterior part of the cortical nucleus are olfactory.

*Cortico-amygdaloid transition area.* The probable homologies of the transition area between the amygdala and the adjacent parts of the piriform cortex have previously been discussed (Meyer & Allison, 1949). It is only necessary to state here that in the human brain two parts of the transition area can be recognized; the smaller anterior part, lying between the prepiriform area and cortical nucleus, appears to receive a considerable accession of olfactory fibres, while the posterior part, which is situated beneath the entorhinal fissure between the cortical nucleus and the entorhinal area, does not in all probability receive any olfactory fibres.

*Central nucleus and nucleus of the stria terminalis.* The central nucleus is comparatively large and rounded, and it is fairly easily defined in the posterior third of the amygdaloid complex.\* Its cells are smaller and stain more lightly than those of the basal complex, which are adjacent to it. The nucleus of the stria terminalis has cells of a similar type; it is small in section and very much attenuated where it lies over the internal capsule, but it expands behind the anterior commissure and extends downwards into the medial preoptic region. The positions of these nuclei are shown in Pls. 1 and 2, figs. 4 and 8. It is not possible, of course, in normal material, to see whether they receive olfactory fibres, but again the analogy with other animals makes it likely that they play some part in olfactory reactions, even though both nuclei are extensive when compared with the relatively small olfactory areas in man.

#### PATHOLOGICAL MATERIAL

Transection of the olfactory tract in the rabbit is followed after a time interval of about a month by marked trans-synaptic atrophy of the pyramidal cells of the prepiriform area (Winkler, 1918; Allison, 1953*b*). Uyematsu (1921) described two human brains with unilateral destruction of the olfactory peduncle in which there was cellular atrophy in the piriform cortex. The brain described in the section on material above, in which the olfactory tract had been accidentally severed on the one side by a leucotomy cut 2 years before death, shows similar changes. On the side of the olfactory tract lesion there is a definite shrinkage and pyknosis of the pyramidal cells of the prepiriform area. The cell bodies are so deeply stained that their nuclei are invisible. This atrophic change corresponds precisely to the limits of the prepiriform area as already described on the side of the transection; on the other side, and in the other olfactory areas on both sides, the cellular architecture appears to be normal. The severity of the trans-synaptic atrophy in the cells of the prepiriform cortex presumably reflects the dependence of this part of the brain upon incoming olfactory impulses, while the sparing of the other areas suggests that they may be concerned with other activities as well as olfaction.

\* Only the most anterior part of the nucleus is shown in Pl. 2, fig. 8.



## COMMENT

The main results of this investigation are indicated in Pl. 1, figs. 1 and 2. They may be summarized briefly by stating that fibres from the olfactory bulb pass through the elongated olfactory crus to join the orbital surface of the frontal lobe. Here the great majority of olfactory tract fibres deviate sharply in a lateral direction (as the lateral olfactory stria) and pass outward to the junction of the frontal and temporal lobes. A thin, superficial band of medullated fibres (the medial olfactory stria) leaves the medial side of the olfactory tract to be expended in the anterior part of the olfactory tubercle; they do not, apparently, reach the septal region of the subcallosal gyrus. A thin sheet of olfactory fibres spreads out on the lateral side of the tract also; it ends first of all over the small dorsal and external parts of the anterior olfactory nucleus and then over the frontal prepiriform cortex. Most of the olfactory tract fibres, however, reach the temporal lobe, where they are only faintly visible macroscopically because they are spread out as a thin superficial layer over a comparatively wide area. Most of these fibres pass anteriorly or medially to terminate in the plexiform layer of the temporal prepiriform cortex. Others travel in a postero-medial direction from the tract and appear to end in the superficial nuclei of the cortico-medial group in about the anterior one-third of the amygdaloid complex. The olfactory component of the anterior limb of the anterior commissure is a small bundle of fine fibres that leaves the olfactory peduncle, passes into the brain above the olfactory tubercle, joins the antero-ventral side of the anterior commissure and probably distributes impulses bilaterally to the nucleus of the stria terminalis and the central amygdaloid nucleus and also to the periventricular and granular layers of the contra-lateral olfactory bulb.

## DISCUSSION

The olfactory areas in the human brain occupy a very similar position to those in the monkey's brain, although the relative increase in size of the neocortex and the entorhinal area in man has brought about some minor specific differences. Thus the frontal prepiriform area and the part of the anterior perforated substance receiving olfactory fibres in the human brain are both relatively smaller than they are in the brain of the monkey, and the olfactory parts of the cortico-medial group of amygdaloid nuclei (mainly the nucleus of the lateral olfactory tract and the medial amygdaloid nucleus) are in man even further reduced in size when compared with the prominent baso-lateral group than they are in the monkey.

The little evidence that is available from other sources fits in well with the location of the primary olfactory area in the gyrus ambiens, or, in other words, the anterior continuation of the hippocampal gyrus. In 1890, Jackson & Beever described a tumour of the right temporo-sphenoidal lobe which had caused episodic disturbances in the form of olfactory seizures; the tumour was sharply circumscribed and the olfactory nerves were intact. Later the 'uncinate fits'—with olfactory hallucinations—came to be well known clinically: they are produced by irritative lesions which always directly or indirectly involve the region of the gyrus ambiens, and destruction of this part of the brain is followed by impairment of olfactory acuity (Frazier & Rowe, 1934).

The most satisfactory neuro-pathological material supports this conclusion also. Uyematsu (1921) has described two brains with unilateral destruction of the olfactory peduncle in which the resulting cellular atrophy is confined to the piriform area, 'lateral to the gyrus circumambiens and anterior to the uncus proper', and this area appears to correspond well to the temporal prepiriform cortex as delimited during the present investigation. Others (Tanaka, 1920; Stewart, 1939) have described degeneration in the cornu Ammonis and dentate fascia in arhinencephalic brains, but these findings are of little value because the cases were epileptics in which the hippocampal regions commonly show gliosis or other changes. Indeed, de Jongh (1927) describes a case with no trace of olfactory bulbs and tracts, and when the cornu Ammonis and dentate fascia were examined histologically they were found to be actually better developed than normal. It is thus unlikely that the hippocampal formation receives olfactory fibres directly, or even indirectly. Electrical stimulation of the hippocampus in conscious human patients does not give rise to any olfactory sensations (Penfield & Erickson, 1941). Moreover, in cases of epilepsy—in which the hippocampus often exhibits pathological changes—there does not appear to be any abnormality in the sense of smell.

#### SUMMARY

The secondary olfactory areas have been delimited in sections through three normal human brains. The olfactory tract fibres appear to end in the antero-lateral part of the olfactory tubercle, the dorsal and external parts of the anterior olfactory nucleus, the frontal and temporal parts of the prepiriform area, the cortico-medial group of amygdaloid nuclei and the nucleus of the stria terminalis. The positions of these areas on the surface of the brain and in representative frontal sections are shown in Pls. 1 and 2. Confirmatory evidence for some of the above conclusions is provided by the localization of the trans-synaptic atrophy in the cells of the prepiriform cortex resulting from accidental transection of the olfactory peduncle.

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## LIST OF ABBREVIATIONS

<i>a.entorh.</i>	entorhinal area	<i>n.amyg.cort.</i>	cortical amygdaloid nucleus
<i>amyg.</i>	amygdala	<i>n.amyg.lat.</i>	lateral amygdaloid nucleus
<i>a.prepir.fr.</i>	frontal part of the prepiriform area	<i>n.amyg.med.</i>	medial amygdaloid nucleus
<i>com.ant.</i>	anterior commissure	<i>n.diag.b.</i>	nucleus of the diagonal band
<i>com.ant.l.ant.</i>	anterior limb of the anterior commissure	<i>n.str.term.</i>	nucleus of the stria terminalis
<i>gyr.hipp.</i>	gyrus hippocampi	<i>pall.</i>	pallidum
<i>gyr.rect.</i>	gyrus rectus	<i>put.</i>	putamen
<i>hipp.</i>	hippocampus	<i>sulcus.rh.</i>	sulcus rhinalis
<i>n.amyg.bas.</i>	basal amygdaloid nucleus	<i>sulcus.semi-ann.</i>	sulcus semi-annularis
<i>n.amyg.cent.</i>	central amygdaloid nucleus	<i>tr.olf.</i>	olfactory tract
		<i>tub.olf.</i>	olfactory tubercle

## EXPLANATION OF PLATES

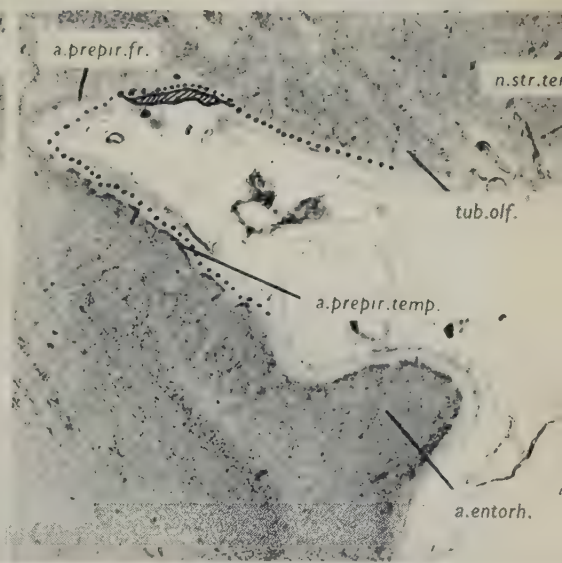
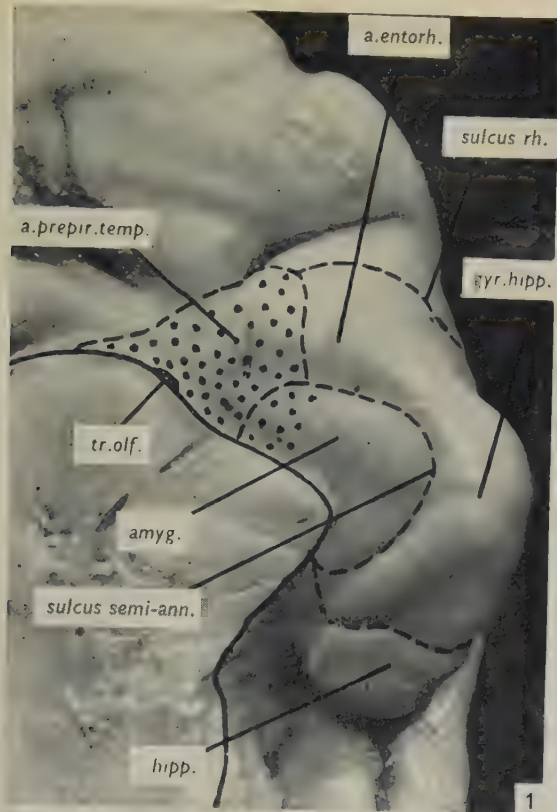
## PLATE 1

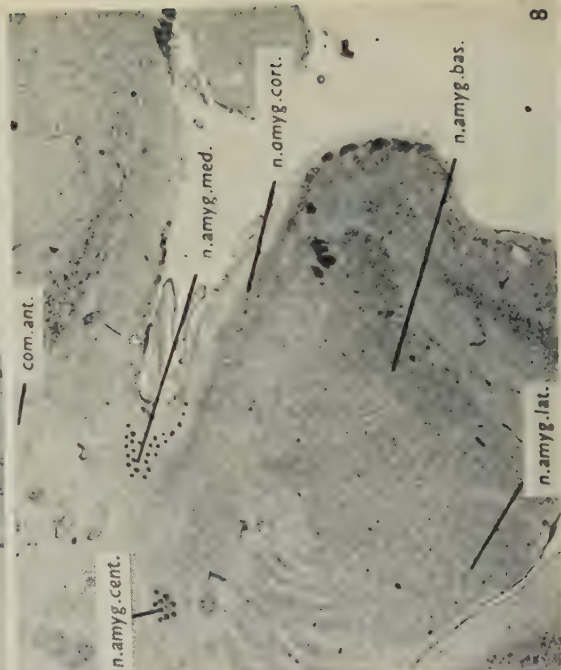
- Fig. 1. Dorsal aspect of the temporal lobe of the human brain (which has been cut away from the frontal lobe),  $\times 2$ , showing the olfactory areas in the region of the uncus. The areas in which olfactory tract fibres probably terminate are indicated by dots.
- Fig. 2. Orbital aspect of the frontal lobe of the human brain (which has been cut away from the temporal lobe),  $\times 1.5$ , showing the position of the basal olfactory areas and the distribution of olfactory tract terminals (dotted).
- Figs. 3 and 4. Representative frontal sections through the olfactory areas of the basal telencephalon of man. Cresyl violet stain,  $\times 7.5$ . The olfactory tract is cross-hatched and the probable situation of olfactory tract terminals is indicated by dots.

## PLATE 2

- Figs. 5-8. Frontal sections through the basal telencephalic areas of the human brain. Cresyl violet stain,  $\times 7.5$ . The olfactory tract is cross-hatched and the areas in which the olfactory tract fibres probably terminate are indicated by dots.







# THE ORIGIN OF THE MAMILLO-THALAMIC TRACT IN THE RAT

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## INTRODUCTION

The gross projection of the mamillary nuclei to the anterior nuclei of the thalamus has been established for many years, and it has been shown with the Marchi technique that the fibres of the mamillo-thalamic tract end in all three elements of the anterior nuclear group (Glorieux, 1929; Le Gros Clark, 1932). The exact origin of the different components of this tract, however, is still uncertain; Kölliker (1896) and Ramón y Cajal (1911) considered that they arise as collaterals of the phylogenetically older mamillo-tegmental tract, whereas van Valkenburg (1912) and Fortuyn (1912) claimed that the two tracts were quite independent. The importance of the mamillo-thalamic tract as one of the two pathways connecting the hypothalamus with the thalamus and cortex has been stressed by Le Gros Clark (1938; Le Gros Clark & Meyer, 1950). In an experimental study of the fornix system (Daitz & Powell, 1954) the anterior thalamic nuclei were on occasion incidentally damaged. By correlating the extent of this damage with the resulting degeneration in the mamillary nuclei information concerning the origin of the mamillo-thalamic tract has been obtained.

## MATERIAL AND METHODS

The brains of twenty albino rats in which unilateral lesions had been placed in the fimbria and preoptic areas were used. The animals varied in age from newborn infants to mature adults, and they were allowed to survive for 1–10 months after operation. The brains were fixed in 70 % alcohol and 2 % acetic acid and embedded in paraffin wax. Serial sections were mounted and stained with Borrel's methylene blue.

## OBSERVATIONS

The extent of the damage to the anterior nuclei of the thalamus was very similar in many experiments; for the purpose of description they may be classified into those in which: (a) the fimbria was completely divided without any thalamic involvement; (b) all the anterior nuclei were completely destroyed; (c) the antero-medial nucleus suffered the major damage; (d) the antero-dorsal and antero-ventral were chiefly involved; and (e) the antero-ventral nucleus was principally involved. Representative examples of each of these groups will be described (Table 1).

Gurdjian's terminology (1927) for the mamillary nuclei of the rat will be followed here; he described a distinct large-celled lateral nucleus and a medial nucleus which is divided into a pars medianus, a pars medialis, a pars lateralis and a pars posterior.

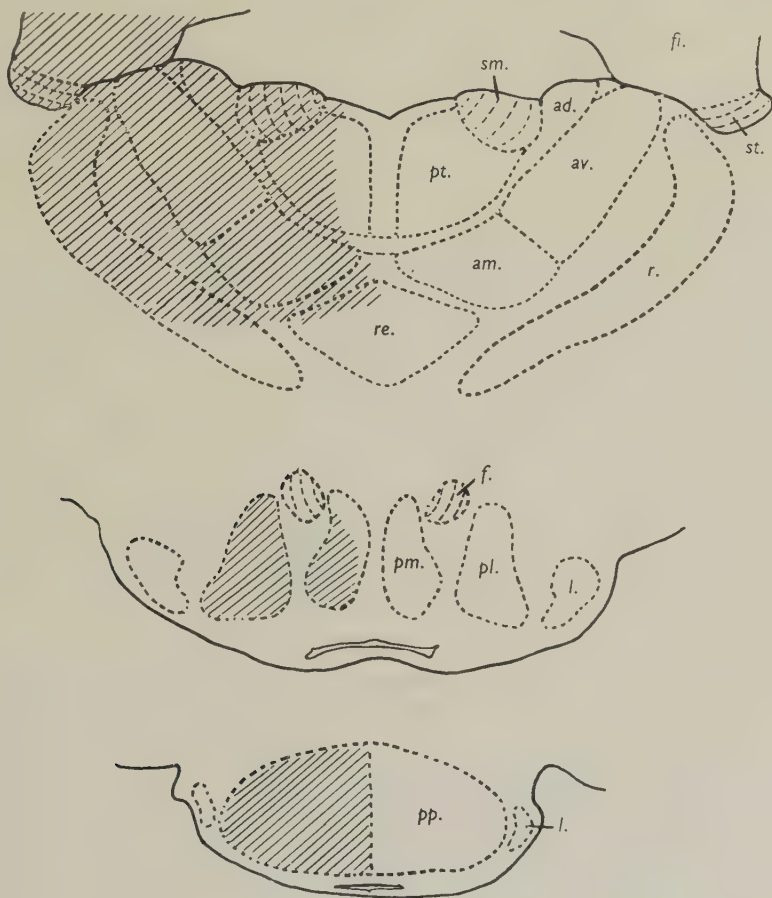
In Exp. D84 a 17-day-old rat was operated upon and allowed to survive for 2 months. The fimbria had been completely divided without any concomitant damage to the thalamus. Careful comparison of all the elements of the mamillary



Table 1. *Extent of damage and the distribution of the resulting degeneration*

Group	No.	Extent of damage				Distribution of degeneration	
		Antero-medial n.	Antero-ventral n.	Antero-dorsal n.		Pars medialis	Pars posterior
<i>b</i>	49	All anterior nuclei completely destroyed				Ventro-lateral two-thirds shows cell loss; and shrinkage and pallor of remaining cells	Severe cell loss; few shrunken pale cells persisting
	54						
	168						
	205						
<i>c</i>	P 3	Completely destroyed	Medial third involved	Unaffected		Ventro-lateral two-thirds shows cell loss; shrinkage and pallor of remaining cells	In ventro-medial third the cells are shrunken and pyknotic
<i>d</i>	48	Lateral margin involved	Completely destroyed	Dorso-lateral two-thirds destroyed		Slight cell loss in lower lateral part	Whole nucleus shows severe cell loss and shrinkage and pallor of remaining cells
	50	Not affected	Lateral margin of both involved		Not affected	Not affected	Almost the whole nucleus shows degeneration but many shrunken cells persist
	57	Lateral margin involved	Both are severely damaged but some cells persist		Not affected	Not affected	
	80	Not affected	Both are completely destroyed		Not affected	Not affected	Both show severe degeneration, but some shrunken cells remain, particularly in dorso-medial parts of each
	82	Not affected	Completely destroyed	Severely damaged but caudally about a third of the nucleus remains	Not affected	Not affected	
<i>e</i>	83	Not affected	Lateral two-thirds severely damaged	Slight involvement of lateral margin	Not affected	Not affected	Middle half is severely degenerate
	150	Not affected	Dorsal one-third and lateral half of the ventral two-thirds destroyed	Dorsal part slightly involved	Not affected	Not affected	Moderate cell loss and shrinkage and pallor of cells in the lateral two-thirds
	170	Not affected	Lateral part involved	Intact	Not affected	Not affected	Dorso-lateral quadrant shows slight cell loss and shrinkage of cells
	197	Not affected	Dorso-lateral two-thirds damaged—more marked anteriorly	Lateral third involved	Not affected	Not affected	Ventro-lateral two-thirds shows cell loss and shrinkage and pallor of cells—more marked caudally
	207	Not affected	The dorso-lateral half is damaged	Lateral margin involved	Not affected	Not affected	Cell loss and shrinkage in the lateral half
	209	Not affected	Dorso-lateral two-thirds damaged	Not affected	Not affected	Not affected	Cell loss and shrinkage of cells in the lateral half
	213	Not affected	Ventro-lateral half damaged	Lateral margin involved	Not affected	Not affected	Cell loss and shrinkage in the lateral third of each

nuclei showed no difference between the normal and operated sides. This, and the other experiments in this group, have been included to obviate the possible criticism that the degenerative changes to be described below may be transneuronal following interruption of one of the principal afferent pathways to the mamillary nuclei rather than to involvement of the anterior thalamic nuclei.



D 168

Text-fig. 1. All anterior thalamic nuclei destroyed. In this and the following text-figures, the extent of the damage to the thalamic nuclei and the resulting retrograde degeneration in mamillary nuclei are indicated by hatching. The outlines have been traced from transverse sections.

In D168, a 7-day-old rat was operated upon and allowed to survive for 8 months. In addition to division of the fimbria all the anterior thalamic nuclei of the left side have been completely destroyed. Severe cellular degeneration was found in almost the entire extent of the medial mamillary nucleus of the operated side (Text-fig. 1). In the ventrolateral two-thirds of the cross-sectional area of the pars

medialis a marked cell-loss has occurred and the remaining cells are all shrunken and stain less intensely than normal. In the dorso-medial third there is no cell loss but a few of the cells appear shrunken. The pars lateralis and the pars posterior have both undergone almost total atrophy, only an occasional pale shrunken cell remaining. The changes are accompanied by a moderate gliosis. The pars medianus and the lateral mamillary nucleus showed no change.

In another experiment, D49, with a similar lesion but with a survival period of 5 weeks the distribution of the degeneration is the same but many more shrunken neurons persist. Further, in this experiment and in others with longer survival periods in which the premamillary nuclei were available for histological examination both the dorsal and ventral premamillary nuclei showed slight degeneration; only a few cells have disappeared but many of the cells were shrunken and less deeply staining.

In Exp. P3 a lesion had been placed primarily in the preoptic areas. This extended back however into the thalamus destroying the ventral anterior nucleus, the whole of the antero-medial nucleus and the medial third of the antero-ventral nucleus; the antero-dorsal nucleus was not involved by the lesion. The animal used was a young adult and was allowed to survive for 7 months after operation. In the mamillary nuclei of the operated side the pars medialis shows the most striking changes. Except for the dorso-medial third, the nucleus showed cell loss and shrinkage and pallor of the remaining cells (Pl. 1, fig. 2). In the pars lateralis and pars medianus there was no change, but in the ventro-medial third of the pars posterior the majority of the cells are shrunken and pyknotic (Text-fig. 2). In the premamillary nuclei a proportion of the cells appeared shrunken and pyknotic. It is apparent from this experiment that the pars medialis projects to the antero-medial nucleus.

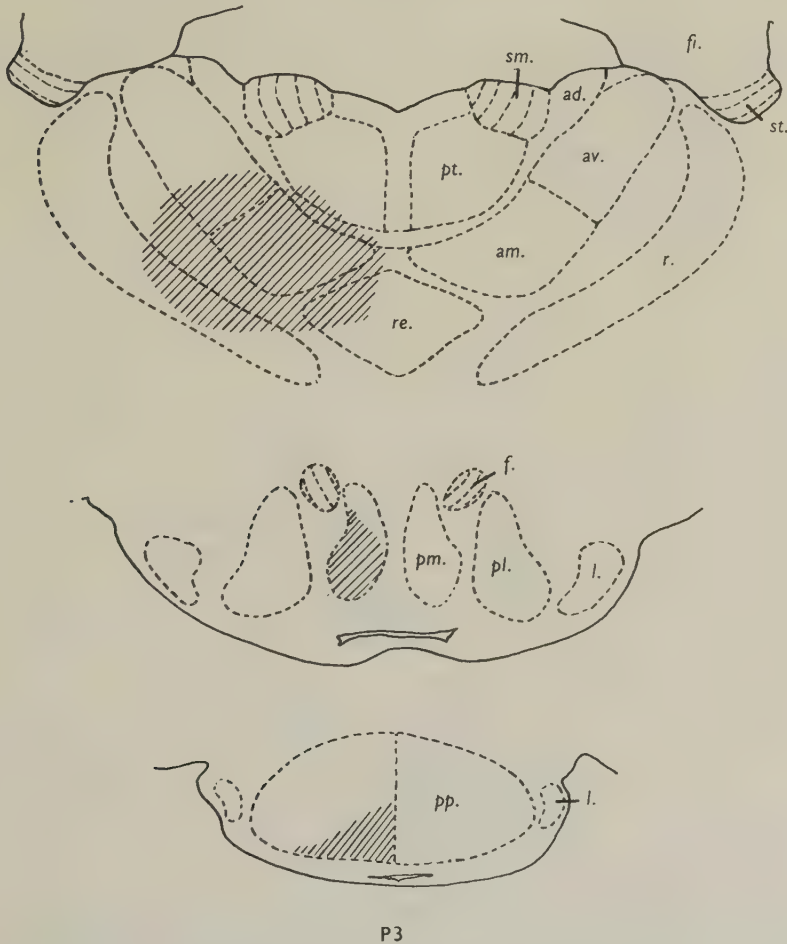
In the majority of the experiments both the antero-ventral and antero-dorsal thalamic nuclei were involved to a greater or lesser extent. A good example of this group is D80 in which the antero-ventral and antero-dorsal nuclei were completely destroyed without involvement of the antero-medial nucleus. The rat was 4 weeks old when operated upon and was allowed to survive for 1 month. In the medial mamillary nucleus of the operated side the pars lateralis and pars posterior are severely degenerated; a marked cell loss has occurred and all the remaining cells are shrunken. The pars medialis and pars medianus are unaffected (Text-fig. 3). This experiment indicates that the pars lateralis and pars posterior are related to the antero-dorsal and antero-ventral nuclei of the thalamus. Further, by correlating the extent of the damage to the antero-dorsal and antero-ventral nuclei in other experiments with the distribution of the degeneration in the mamillary nuclei it is inferred that the pars lateralis projects to the antero-dorsal nucleus and that the pars posterior projects to the antero-ventral nucleus. This is confirmed by the final group of experiments of which D197 is an example.

In this experiment fimbrial section was performed on an adult rat and the survival period was 8 months. The incidental damage to the thalamus involved the dorsal and lateral parts of the antero-ventral nucleus and the lateral third of the antero-dorsal nucleus. In the medial mamillary nucleus the resulting degeneration was found in the lower lateral third of the pars lateralis and in the ventro-lateral two-



thirds of the pars posterior; in these areas marked cell loss had occurred and the persisting cells were shrunk and stained less deeply than normal (Text-fig. 4).

In all the experiments in which the antero-ventral and antero-dorsal nuclei were only partially involved degeneration was localized to discrete portions of the pars posterior and pars lateralis, and from this it appears that there is a medio-lateral

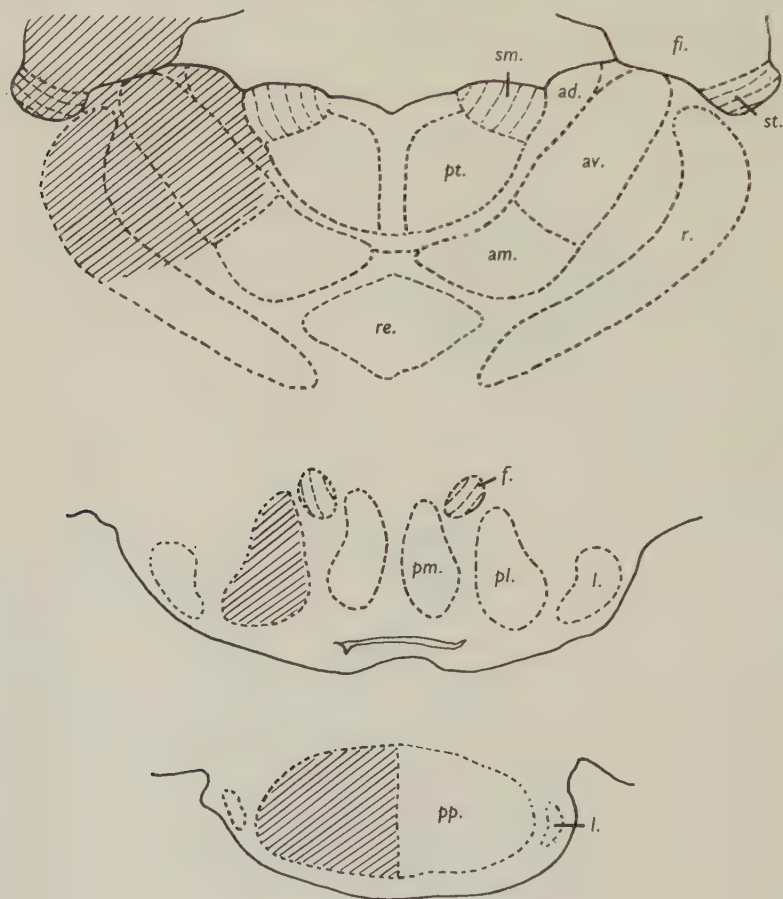


Text-fig. 2. The antero-medial nucleus has been completely destroyed with partial involvement of the antero-ventral nucleus.

organization in the projection of these elements in the sense that medial parts of the mamillary nuclei project to the medial parts of the corresponding anterior nuclei and vice versa. Further, in some experiments there is evidence to suggest that the rostral parts of these two subdivisions of the medial mamillary nucleus project to the caudal parts of the corresponding anterior nuclei and vice versa. It should be pointed out that this is only a tentative conclusion, and more material is necessary to establish this point.

## DISCUSSION

So far as is known, the precise origin of the mamillo-thalamic tract in respect of the different components of the mamillary complex has not been demonstrated experimentally. The results described here show conclusively that in the rat this tract has no origin from the large-celled lateral mamillary nucleus (nucleus



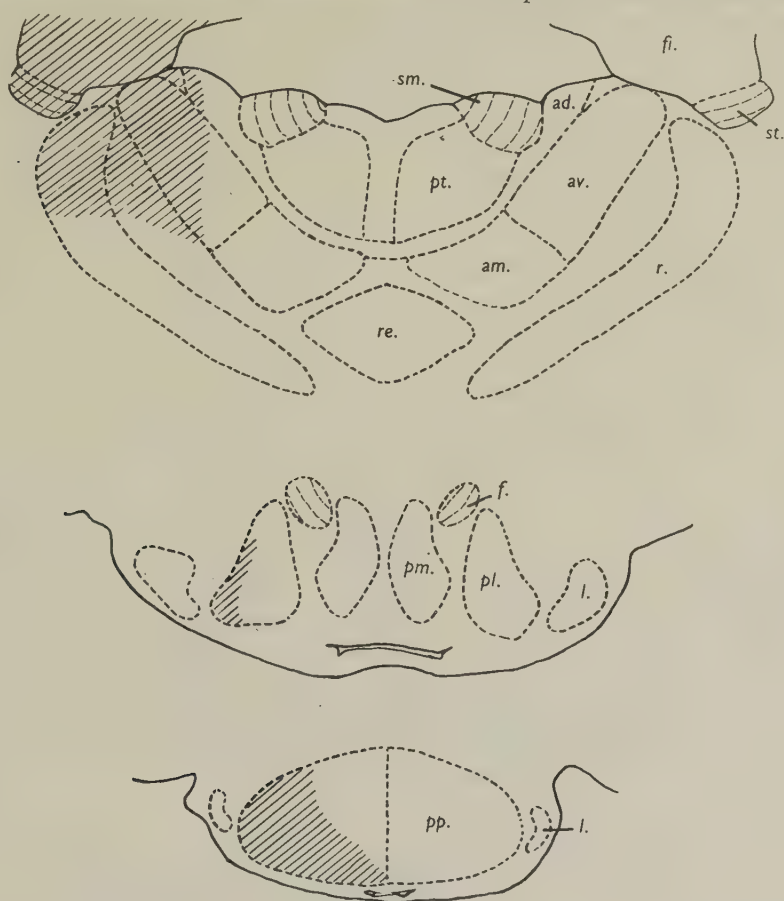
D 80

Text-fig. 3. The antero-dorsal and antero-ventral nuclei have been destroyed without involvement of the antero-medial.

intercalatus in the human brain described by Le Gros Clark, 1938), nor from the pars medianus of the medial mamillary nucleus. Our findings indicate that it arises from the pars lateralis, pars posterior and the ventro-lateral two-thirds of the pars medialis and possibly in part from the dorsal and ventral premamillary nuclei.

It should be noted that even after survival periods of 10 months a small proportion of shrunken cells persist in the affected elements and more particularly in the pars medialis. The persistence of these cells may be due to the presence of intact collaterals, and this is of interest in view of the opinion of Kölliker (1896) and

Ramón y Cajal (1911) that the fibres of the mamillo-thalamic tract arise as collaterals of the mamillo-tegmental tract. However, van Valkenburg (1912) found retrograde cell degeneration localized to the dorsal part of the medial mamillary



D 197

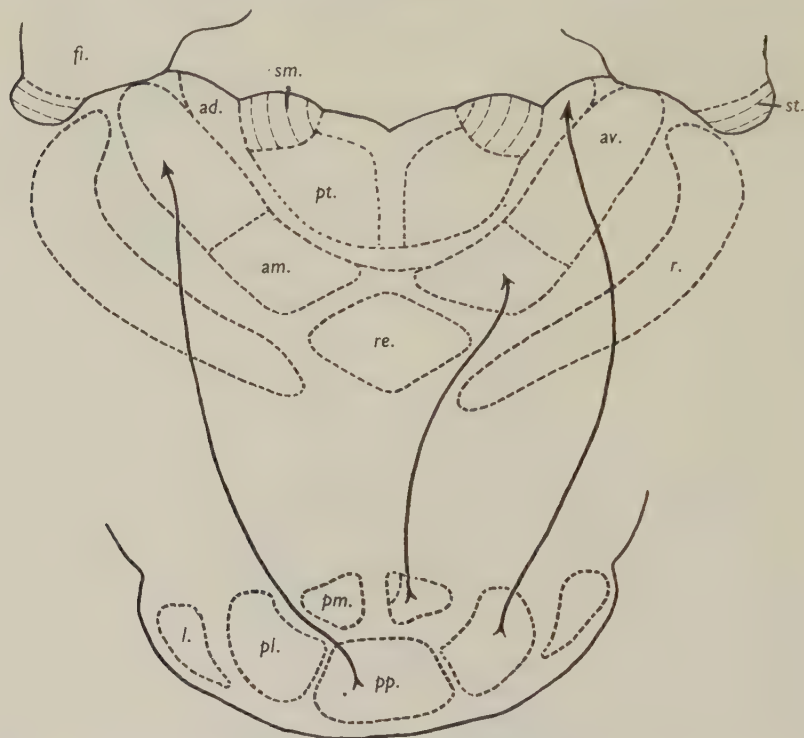
Text-fig. 4. The antero-ventral nucleus has suffered the principal damage.

nucleus after section of the mamillo-tegmental tract. Our findings are in close agreement with this as the dorso-medial part of the pars medialis of the medial mamillary nucleus always remained virtually unaffected even when all the anterior thalamic nuclei had been completely destroyed.

Not only has the origin of the mamillo-thalamic tract in the rat been established, but a precise projection from each subdivision of the medial mamillary nucleus to each element of the anterior thalamic nuclei has been shown to exist. The ventro-medial two-thirds of the pars medialis (and possibly the dorsal and ventral pre-mamillary nuclei) are connected with the antero-medial nucleus, the pars lateralis with the antero-dorsal nucleus and the pars posterior with the antero-ventral nucleus (Text-fig. 5). Within this nuclear projection there is some evidence which indicates a medio-lateral and rostro-caudal organization.



It has been known for some time that the anterior nuclei project to the cortex of the cingular gyrus (Le Gros Clark, 1932), and Rose & Woolsey (1948) have shown that in the rabbit and cat each of the anterior nuclei projects to a distinct architectonic field. Taken in conjunction with the present observations, it is apparent that not only are the different anterior nuclei related to distinct cortical fields but



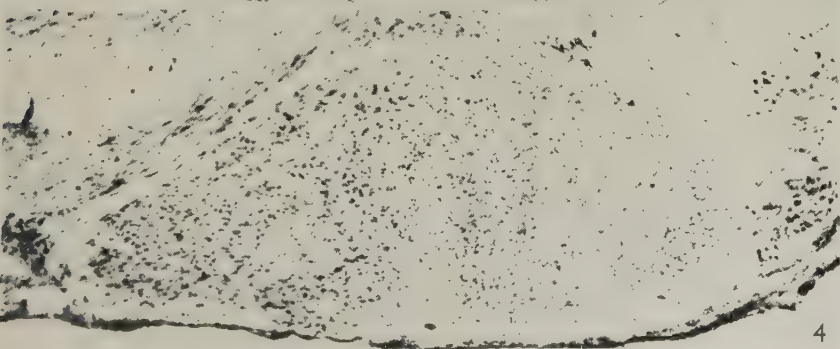
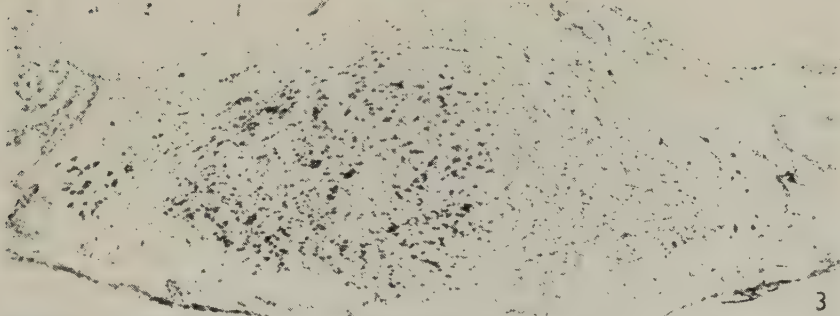
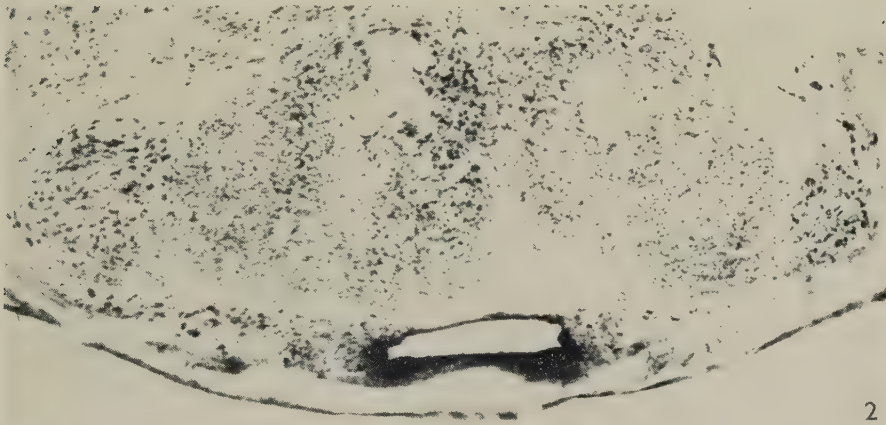
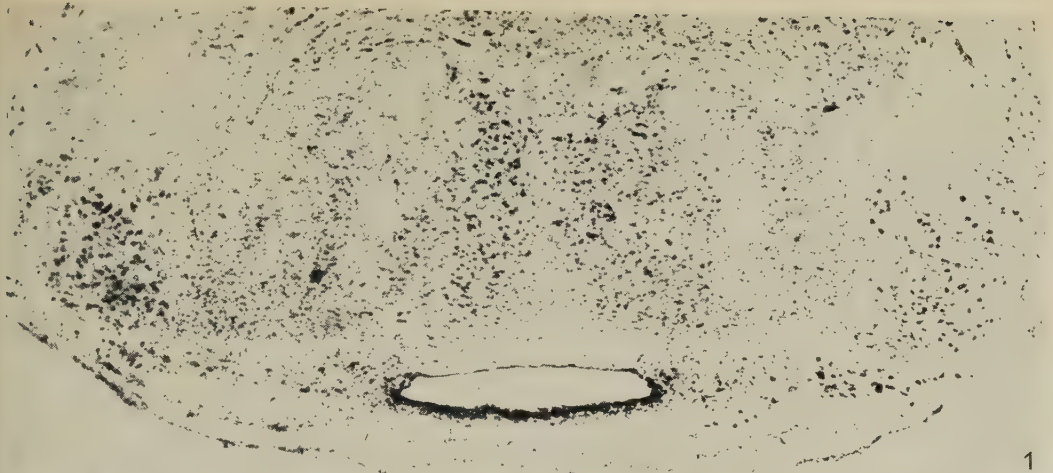
Text-fig. 5. The projection of the individual elements of the medial mamillary nucleus (traced from a horizontal section) to the three anterior nuclei.

that each element of the medial mamillary nucleus is likewise connected with a different subdivision of the cingular gyrus: i.e. pars medialis with the anterior cingular cortex (area 24 of Brodmann), the pars posterior with the posterior cingular cortex (area 23) and the pars lateralis with the retrosplenial area (area 29). This relationship may be of significance in view of the recent physiological and clinical work on the cingular gyrus (see Kaada, 1951, 1953; Fulton, 1951).

#### SUMMARY

1. The origin of the mamillo-thalamic tract in the rat has been studied by the method of retrograde cell degeneration following damage to the anterior nuclei of the thalamus.

2. The tract arises from all elements of the medial mamillary nucleus (except the pars medianus) and possibly from the premamillary nuclei. No contribution is made by the lateral mamillary nucleus.







3. There is a precise projection from each element of the medial mamillary nucleus to each of the anterior thalamic nuclei: the pars medialis projects to the antero-medial nucleus, the pars lateralis to the antero-dorsal nucleus and the pars posterior to the antero-ventral nucleus.

This work was done while one of us (T.P.S.P.) held a Medical Research Council Fellowship in Clinical Research.

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#### EXPLANATION OF PLATE

- All the sections were stained with methylene blue and the magnification throughout is  $\times 58$ .
- Fig. 1. Transverse section of mamillary nuclei in H57 to show retrograde degeneration in the pars lateralis only.
- Fig. 2. Transverse section of mamillary nuclei of P3 to show degeneration confined to the ventro-lateral two-thirds of the pars medialis.
- Fig. 3. Transverse section of pars posterior of the medial mamillary nucleus of D49 to show complete cellular atrophy on the operated side.
- Fig. 4. Transverse section of pars posterior of the medial mamillary nucleus of D150 to show degeneration mainly in the lateral half of this element in the operated side.

In some of these sections there is apparent cell loss in the lateral mamillary nucleus, which is due to slight obliquity of the sections.

#### LIST OF ABBREVIATIONS

<i>ad.</i>	antero-dorsal nucleus	<i>pm.</i>	pars medialis
<i>am.</i>	antero-medial nucleus	<i>pp.</i>	pars posterior
<i>av.</i>	antero-ventral nucleus	<i>pt.</i>	parataenial nucleus
<i>fi.</i>	fimbria	<i>r.</i>	reticular nucleus
<i>f.</i>	fornix	<i>re.</i>	nucleus reuniens
<i>l.</i>	lateral mamillary nucleus	<i>sm.</i>	stria medullaris
	medial mamillary nucleus:	<i>st.</i>	stria terminalis
<i>pl.</i>	pars lateralis		

## A METHOD OF ASSESSING SKELETAL MATURITY FROM RADIOGRAPHS

A REPORT FROM THE OXFORD CHILD HEALTH SURVEY\*

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It has long been realized that skeletal development is divisible into two components, increase in size and increase in maturity. Although closely integrated in the healthy child, each follows its own individual pattern. Increase in size is relatively easy to assess; skeletal maturation, however, is not only elusive of measurement, but is also difficult to define. It is usually accepted as being the metamorphosis of the cartilaginous and membranous skeleton of the foetus to the fully ossified bones of the adult. It can be studied conveniently by X-ray.

### THE LITERATURE

The hand (including the wrist) has received most attention in the literature, both because it is easy to radiograph, and because it includes a wide range of bones suitable for study. The work of Rotch (1908, 1909), Flory (1936), Todd (1937) and Greulich & Pyle (1950) suggests that this region offers a fair index of the maturity of the entire skeleton of the healthy child. The most popular method of assessing maturity, therefore, has been to base comparison on a series of films which are typical of the various age groups. Such pictorial standards have been published by Wilms (1902), Rotch (1909), Englebach & McMahon (1924), Siegert (1935), Flory (1936), Todd (1937), Vogt & Vickers (1938), Greulich & Pyle (1950) and Mackay (1952). However, this 'inspectional' method involves considerable subjective error. To eliminate the latter, efforts were made to assess maturity by measuring the size of the shadows of various bones on the radiograph (Baldwin, 1921; Lowell & Woodrow, 1922; Carter, 1926; Baldwin *et al.* 1928; Sawtell, 1929; Prescott, 1933; Cattell, 1934; West, 1936). Such techniques were little used outside the centres in which they were devised because they were slow, cumbersome and inaccurate. Nevertheless, they had the great advantage that they offered skeletal maturity its own yardstick (Shuttleworth, 1938).

A third method has been evolved which entails radiographing all the joints on one side of the body, and counting the number of centres which have ossified; and later the number of epiphyses which have fused (Sontag, Snell & Anderson, 1939; Sontag & Lipford, 1943; Lurie, Levy & Lurie, 1943). This system involves many radiographic films and is therefore expensive; it also ignores the structural changes which occur in the epiphyses between their first appearance and their fusion with the diaphyses.

\* This Survey has been financed by grants from the Medical Research Council and the Nuffield Provincial Hospitals Trust.

### THE DISADVANTAGES OF THE INSPECTIONAL TECHNIQUE

Of those described, the inspectional technique alone is generally used. The *Atlas* of Todd (1937), and its revision by Greulich & Pyle (1950) are the standard works of reference. These offer an excellent method for rapid assessment of maturational status suitable for general clinical purposes, but they do not permit an accurate evaluation of any film for the following reasons:

(1) *A fixed pattern of first appearance and subsequent development of centres is presupposed.* A standard film is published for each age group and, if these are studied serially, it is found that the centres appear in a certain order, and their subsequent development proceeds in a fixed pattern. There is, however, a considerable amount of evidence to show that a wide range of normal variation exists in the pattern of ossification, and that this variation is genetically determined (Pryor, 1908, 1936, 1939; Buschke, 1934, 1935; Reynolds, 1943). What is more, there is reason to believe that certain illnesses alter the order of appearance of the bones (Todd, 1930, 1933; Francis, 1939; Buehl & Pyle, 1942). It follows that many instances occur when the film to be assessed shows a pattern of ossification which is radically different from that of the standard. Assessment in these cases necessarily introduces a subjective error.

(2) *There is too long a time interval between the standard films.* During the greater part of childhood the standard films are placed 6 months apart. This coarse grouping is essential to the method because it is only if there is a very sharp distinction between two successive standards that any attempt can be made to overcome the pattern differences described in § (1) above. If the time interval between the standards is reduced, for instance to 1 month, the film of a child whose pattern of ossification differed radically from that shown in the *Atlas* might bear an equal resemblance to several successive standard films. In this way the subjective error in assessment would be further increased.

There are two more objections to the Inspectional Technique:

(3) *The necessity for a set of standards for each sex.* It is a commonplace that the female matures more rapidly than the male. It follows that at any age the two sexes will have reached different maturational levels, and therefore will require separate sets of standards. In other words, the term 'skeletal age 30 months' calls to mind no radiographic picture, unless it is qualified by the sex to which it applies.

(4) *The use of time as a yardstick.* Skeletal maturation is a process as distinct in itself as that of growing bigger or growing heavier. Therefore, just as growth is measured in inches and pounds, maturation should have units of its own. To speak of the mean skeletal maturity status of a group of children aged  $2\frac{1}{2}$  years as 'skeletal age 30 months' is no more reasonable than to speak of their mean weight as being 'ponderal age 30 months'. Just as every child has its individual pattern of weight increase so it has its individual pattern of maturation. Both of these correlate with time, but neither correlates so closely that it can be looked upon as 'happening in months and years', for that, in fact, is what the concept 'skeletal age' implies. This concept (or misconception) has been an important factor in impeding the progress of understanding of this field.



For these reasons an attempt has been made to devise a method of assessing maturity in which:

(1) Every round bone and epiphysis can make its own contribution to each assessment, and so evaluation of a film can be made regardless of the pattern in which ossification is occurring.

(2) Small increases of maturity are recorded.

(3) Maturation is given a yardstick of its own, the units being Oxford Maturity Units.

(4) The same standards are used for both sexes, so that a direct comparison can be made between the unit status of any boy and girl.

THE PRINCIPLES OF THE OXFORD METHOD

Todd's greatest contribution to this field of study was a description of the exact shadow changes in a radiograph which indicated increasing skeletal maturity. He concentrated his attention on the growing ends of the long bones: 'successive changes in outline of shaft ends and in contour of epiphysial ossification centres' (1937). Greulich & Pyle (1950) have, by illustrating the denominators of maturity in the round bones of the carpus, added to Todd's work.

In the Oxford Survey it was decided that a unit should be awarded to a bone as each distinct shape change made itself manifest, and in this way the sum total of units scored by a bone at any stage in its development would be an exact measure of its maturity. This technique is equally applicable to any part of the body, provided that the maturity denominators of the bones are clearly recognized. In the present paper the maturity indicators recognizable in the hand and knee of a healthy group of British children between the ages of 6 months and 5 years are described.\* The indicators accepted in the hand and wrist are based upon those described by Greulich & Pyle (1950); they were chosen because they were easily recognized in a large number of films (see Fig. 1).

\* For details of recruiting and composition of the Oxford Child Health Survey see Ryle (1948) and Stewart & Russell (1952).

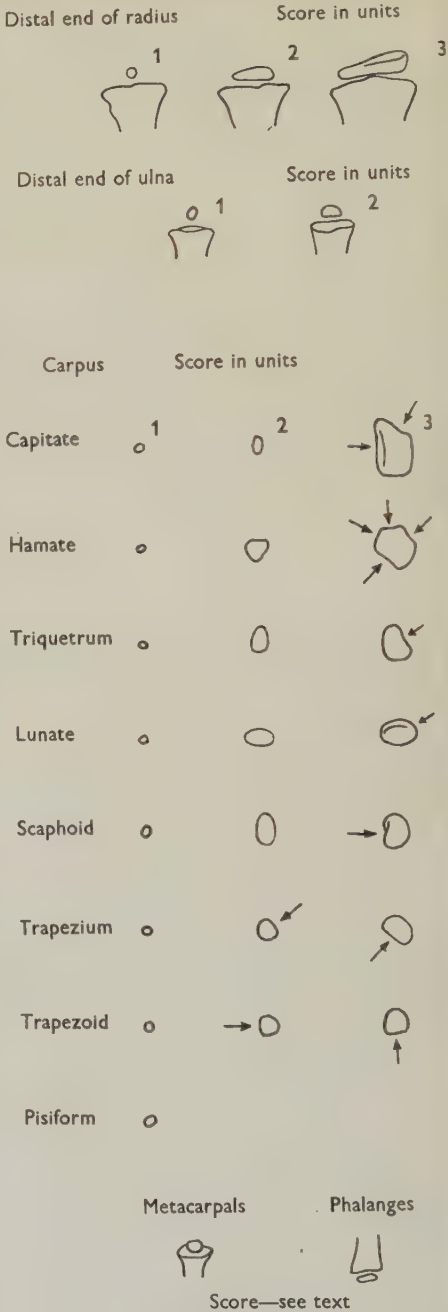


Fig. 1. For legend see p. 501 (after Greulich & Pyle).

Fig. 1. Denominators of maturity—the hand (after Greulich &amp; Pyle)

Score in units			
	1	2	3
Distal end of radius	Primitive rounded centre	Broad laterally narrow medially	Volar margin of distal surface visible as a line
Distal end of ulna	Primitive rounded centre	Flat proximally rounded distally	
<i>Carpus</i>			
Capitate	Primitive rounded centre	Oval in appearance	Flattening in articulation with second metacarpal, and in articulation with the hamate
Hamate	Primitive rounded centre	Triangular shape	Evolution of surfaces articulating with triquetrum, metacarpals V and IV, and capitate
Triquetrum	Primitive rounded centre	Piriform shape	Surface articulating with lunate becomes distinct
Lunate	Primitive rounded centre	Oval shape	Volar surface of capitate articulation defined as a line
Scaphoid	Primitive centre (occasionally somewhat oval)	Definite ovoid	Surface articulating with capitate flattened
Trapezium	Primitive rounded centre	Slight flattening of surface articulating with first metacarpal	Slight flattening of surface articulating with scaphoid
Trapezoid	Primitive rounded centre	Slight flattening of surface articulating with capitate	Slight flattening of surface articulating with scaphoid
Pisiform	Primitive rounded centre	No further development noted in present series	
Metacarpals } Phalanges }	Presence of epiphyses	Score. See text.	

The only previous work of reference known to the author for the knee is a pioneer monograph by Sick (1902), which deals with the subject very superficially. The suggested indicators shown in Fig. 2 have been selected because they were consistently observed in about 1200 serial antero-posterior films of this joint. Fig. 1 represents the bones of the left hand on a postero-anterior film and Fig. 2 the left knee on an antero-posterior film.

#### THE METHOD OF COMBINING THE INDIVIDUAL BONE SCORES TO INDICATE THE OVERALL MATURITY OF THE CHILD

The question of whether or not the maturational status of a child is accurately reflected by the sum total of the individual scores of all its bones raises some questions which, in the present state of knowledge, cannot be answered. In the first place, there is reason to believe that round bone, and epiphyseal ossification do not proceed at equal rates in all children. In other words, one healthy group may show relatively advanced development in the carpus and tarsus, whilst the ossification of their epiphyses is somewhat behind average. In another group the reverse may be true (Sawtell, 1929; Robinow, 1942; Buehl & Pyle, 1942; Schmid, 1949). It is therefore uncertain whether the maturity scores of these two types of bone are a measure of the same process. If there were two processes it might not be legitimate to add the round bone and epiphyseal scores together.

The next question that arises is whether the total scores for one anatomical area should be added to those from another. Do total hand points plus total knee points

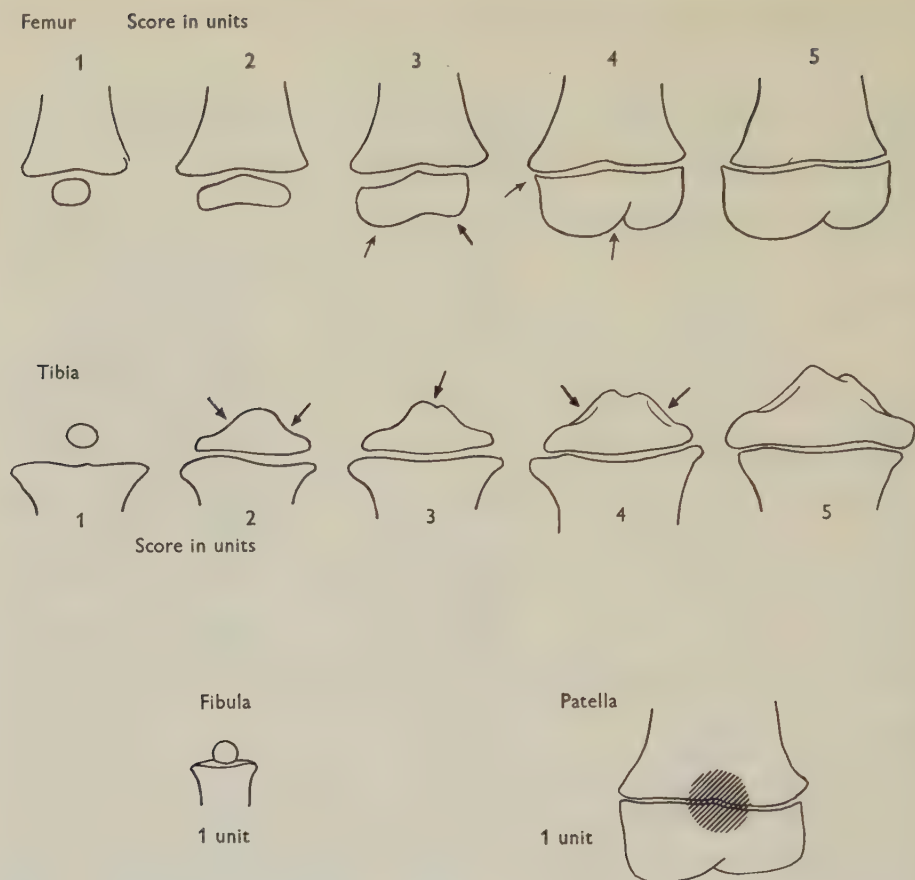


Fig. 2. Denominators of maturity—the knee

Femur	Score 1	Score 2	Score 3
	Rudimentary centre usually rounded	Epiphysis more elongated and somewhat 'banana shaped'	Condyles visible as definite entities
	Score 4	Score 5	
	(a) line running from medial condyle into bone and/or (b) medial proximal corner of epiphysis becoming differentiated as a sharp point	Epiphysis as broad as diaphysis (checked by measurement)	
Tibia	Score 1	Score 2	Score 3
	Rudimentary centre; usually rounded sometimes triangular	Definite triangular shape with tendency to indentation on proximal surfaces	Development of intra-condylar eminence (attachment of ligaments). Higher on medial side
	Score 4	Score 5	
	Surface of tibial table begins to show itself as lines	Epiphysis as wide as diaphysis (checked by measurement).	
Fibula	Score 1		
Patella	Presence of epiphysis Seen as a denser shadow through lower part of femur		



give a more accurate picture of maturational status than considering one area alone? If there is a considerable difference between the scores of two regions, must this difference in itself be taken into account? That such differences exist has been shown (Sontag & Lipford, 1943; Mann, Driezen, Pyle *et al.* 1948), but these authors do not agree as to why they exist. In the face of these difficulties it is essential that arbitrary assumptions are made, with the reservation that these must be revised as knowledge of the subject advances. A pilot study of ninety-seven of the Oxford children (forty-five boys and fifty-two girls) was based on the following assumptions:

- (1) That the hand and knee should be treated separately.
- (2) That round bone and epiphyseal ossifications are facets of the same process, and that it is therefore justifiable to add their scores.

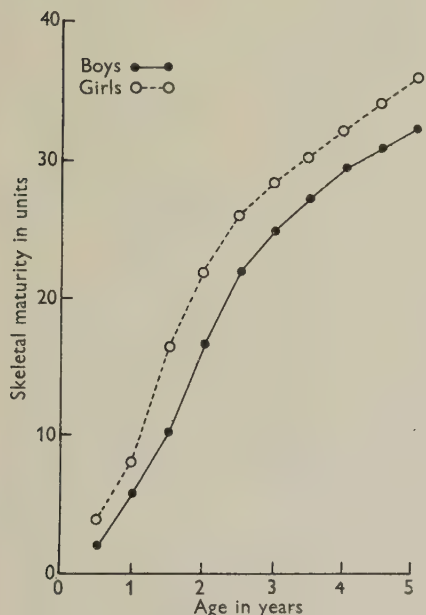


Fig. 3. The hand—gross maturity.

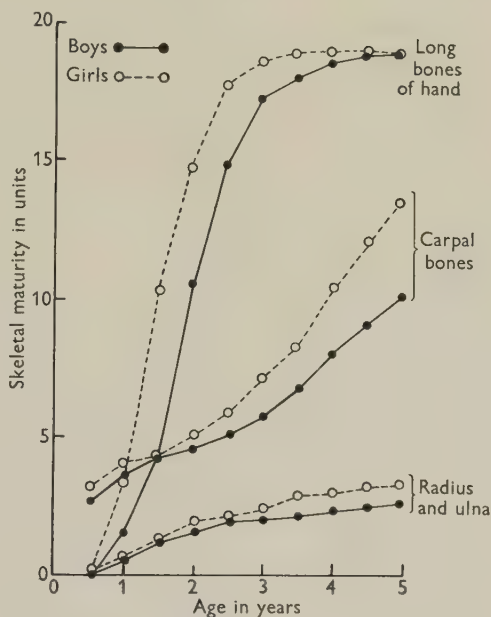


Fig. 4. The hand—maturity analysed.

(3) Should a rectilinear relationship not be found between skeletal maturation and age in respect of either region, that it is justifiable to contrive such a relationship. This assumption was made because the study of increments is greatly simplified if they are even throughout the period under observation.

It must be emphasized that these assumptions are recognized as being the basis of an experimental method of computation, and that each will be revised as and when advancing knowledge indicates that a revision is necessary.

*The hand.* Fig. 3 shows the mean score for the hand in Oxford Maturity Units, plotted against age. The lines are curved for both sexes. If the totals are broken down into their contributing parts: (i) the epiphyses of the long bones of the hand, (ii) the bones of the carpus, and (iii) the distal epiphyses of the radius and ulna, it becomes plain that the inequality of increment in each sex is due to the rapid appearance of the epiphyses of the long bones of the hand (Fig. 4). Equal increments

(in keeping with assumption 2) can therefore be achieved either by awarding further points to these bones before the age of 5 years (thus making the curve steeper), or by scaling down their contribution to the total score. The first technique was attempted and abandoned because the only constant maturity indicator for these bones in every child during the age range under study, was the first appearance of the epiphysis. Therefore the contribution of these bones was scaled down. The distal and proximal phalanges of the thumb each scored full weight, i.e. one unit. Each

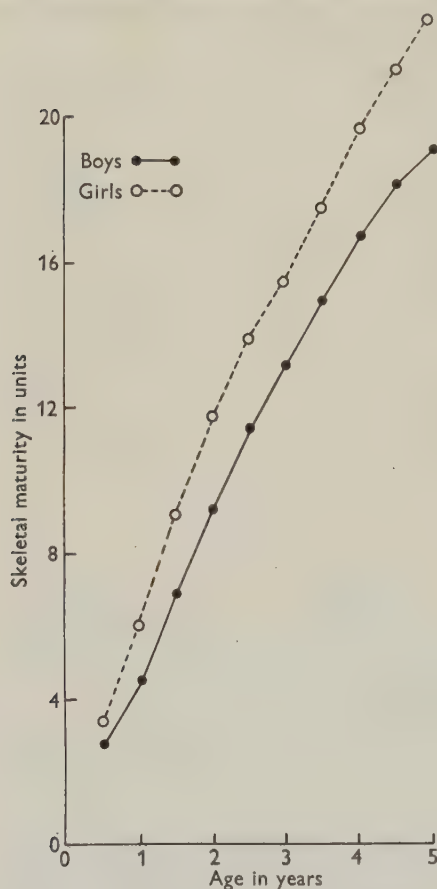


Fig. 5. The hand—corrected maturity.

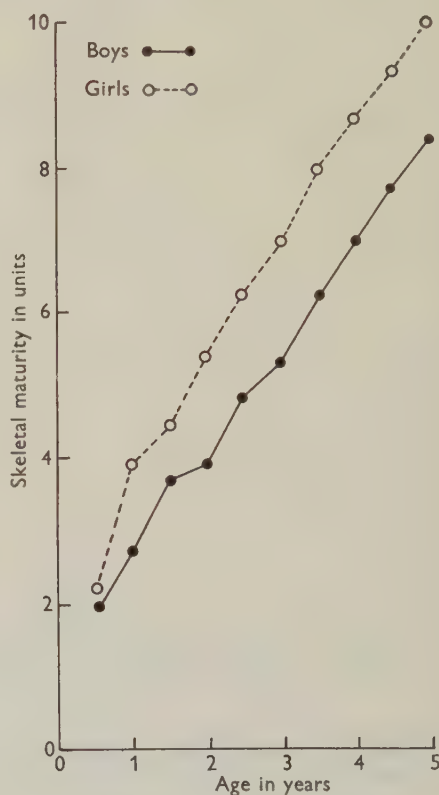


Fig. 6. The knee—maturity.

row of phalanges of the fingers scored one unit when they were complete, each epiphysis contributing 0.25 unit to the total score. The five metacarpals also contributed one unit between them, each being valued at 0.2 unit. In this way the overall contribution of the long bones of the hand was reduced from 18 to 6. The relationship of this corrected score with age is shown in Fig. 5 and Table 1. A reasonably straight line has been contrived.\*

\* The hand films have also been assessed against the standards of Todd (1937). This has been done so that the data are directly comparable with those of the American Growth Studies (Acheson & Hewitt, 1954).

*The knee.* No mathematical adjustment was necessary for this region. The mean scores (Table 2, Fig. 6) show that apart from irregularities during the early years the increments were fairly constant. These irregularities are due to the fact that some difficulty was experienced in defining satisfactory maturity indicators during this period.

# DISCUSSION

It is now necessary to examine the efficiency of this technique. Sawtell (1929) stated that a measure which claims to assess skeletal maturity should correlate with height and with weight, and that it should demonstrate the precocity of the female. The

Table 1. *Mean hand score in Oxford Maturity Units by age and sex*

Age (years)	Boys		Girls	
	Mean	S.D.	Mean	S.D.
$\frac{1}{2}$	2.7	0.9	3.3	1.2
1	4.4	1.1	5.9	1.5
$1\frac{1}{2}$	6.8	1.4	9.0	1.6
2	9.1	2.4	11.7	2.5
$2\frac{1}{2}$	11.3	2.1	13.8	2.1
3	13.1	2.2	15.4	2.7
$3\frac{1}{2}$	14.9	2.2	17.5	3.1
4	16.5	1.9	19.4	3.1
$4\frac{1}{2}$	17.8	1.9	21.0	3.4
5	19.0	2.0	22.8	3.2

Table 2. *Mean knee score in Oxford Maturity Units by age and sex*

Age (years)	Boys		Girls	
	Mean	S.D.	Mean	S.D.
$\frac{1}{2}$	2.0	0.1	2.2	0.7
1	2.7	0.3	3.9	0.6
$1\frac{1}{2}$	3.7	0.8	4.4	0.9
2	3.9	0.3	5.3	0.9
$2\frac{1}{2}$	4.8	0.6	6.2	1.2
3	5.2	0.8	6.9	1.4
$3\frac{1}{2}$	6.1	0.8	7.9	1.1
4	6.9	0.9	8.6	1.2
$4\frac{1}{2}$	7.6	0.9	9.2	0.9
5	8.3	0.9	9.9	1.2

present technique fulfils these three criteria. Flory (1936) wrote 'the critical test of a measure is the degree to which it predicts the characteristic to be measured rather than the degree to which it is related to other measures'. It is not yet possible to use the present technique to predict the time at which final maturity will be attained. However, this test must be applied as soon as the material for older subjects is available. It is acknowledged that sexual and skeletal maturity are very closely correlated (Abernethy, 1925; Richey, 1937; Shuttleworth, 1937, 1938; Buehl & Pyle, 1942, etc.) and so a further test will be the accuracy with which puberty can be predicted. At the moment its acceptance must depend on its compliance with the requirements of Sawtell (1929) and the fact that the maturation changes which it assesses are closely analogous to those described by acknowledged authorities (Todd, Greulich and Pyle).



When the technique has been worked out for the entire period of maturation it will probably be convenient to consider the maturational status of any bone or region in terms of percentages. For instance, the hand of a child may be described as being 34 % mature and its knee as 37 % mature. Not only would this enable a comparison to be made between the various parts of the body, but it is a statement which is easily intelligible because morphological maturity, the 100 % level, is inevitable in the healthy person (Krogman, 1949). In addition, the work of Bayley (1943*a*, *b*, 1946, 1952) suggests that such a statement may be of value in the prediction of final height.

#### SUMMARY AND CONCLUSIONS

Existing methods of assessing skeletal maturity are reviewed, and their shortcomings are discussed. A new method is suggested which is based on the recognition of maturity indicators described by acknowledged authorities. Details of the method are given for the hand and knee during the first 5 years of life; however, the technique may be applied to any part of the body throughout the developmental period. The necessity for considering skeletal maturity in units other than time is emphasized, and it is suggested that when the technique has been worked out for the entire developmental period it may be logical and convenient to express all skeletal maturity readings as percentages.

I should like to express my gratitude to Dr F. H. Kemp and to Dr Alice Stewart for their advice and criticism; and to Miss McLarty and Miss Jeremy for help with the figures.

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## SQUATTING FACETS ON THE EUROPEAN TALUS

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It has been suggested that the so-called squatting facets found upon the neck of the talus and the lower end of the tibia in many Oriental races provide evidence for the inheritance of acquired characters, since they are present also in the Oriental foetus (Charles, 1894; Wood Jones, 1944). Sewell (1904) disputed this contention on the grounds that 'these facets occur in the foetus of the European, and probably in all other races, whether the facets are found to be present in the adult or not'. Unfortunately, as Inkster (1927) has pointed out, several distinct facets have been described in the literature, and the subject is further confused by the lack of an agreed terminology. Moreover, although many series of adult tali have been reported there has been no comparable study of foetal material. Most workers have examined dry tali; with these it is sometimes impossible to determine whether a smooth area on the neck of the talus is in fact an articular facet.

The present investigation is concerned with two aspects of the problem: first, to define the various facets that occur upon the neck of the human talus; secondly, to study their incidence in a series of adult and foetal European tali.

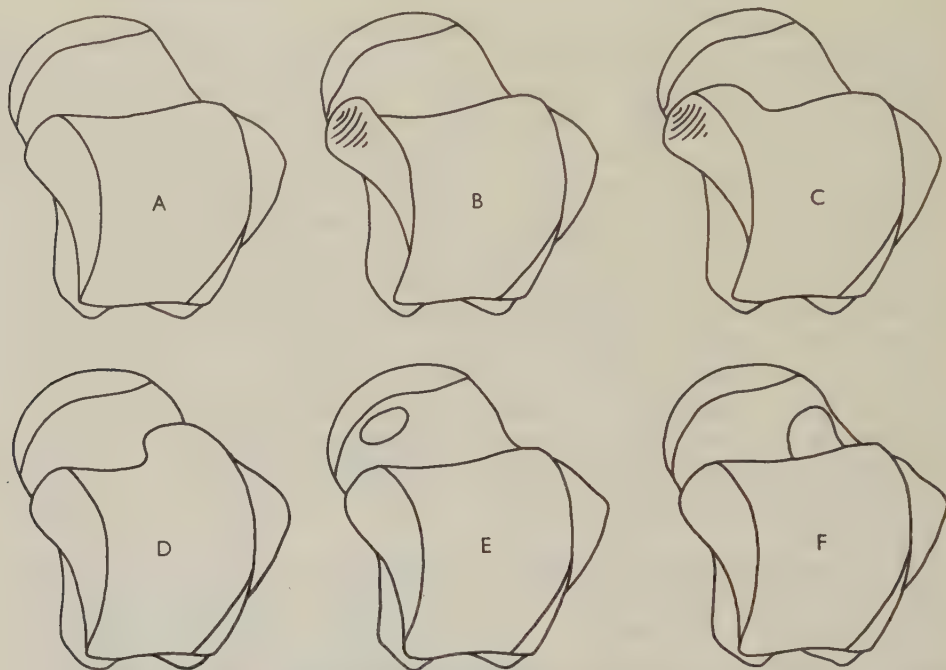
The main types of articular facet that have been described by previous workers are as follows:

(a) A forward prolongation of the comma-shaped medial articular surface of the talus beyond the anterior margin of the trochlea (Charles, 1893). When of large size, this surface is curved medially at its anterior end. The corresponding facet on the tibia is rounded, and cartilage is found covering part of the anterior as well as the lateral aspect of the medial malleolus (Text-fig. 1B).

(b) A rectangular area, covered with articular cartilage, upon the medial side of the upper surface of the neck of the talus has been incorrectly described as a squatting facet (Parker & Shattock, 1884). This area 'which is essentially a prolongation of the trochlea on its medial side, has a surface which continues the line of curvature of the trochlea and must therefore come into contact with the under surface of the lower end of the tibia during [dorsi-] flexion of the ankle and not with its anterior margin' (Inkster, 1927). Since there is no corresponding facet on the tibia, this prolongation is not a true squatting facet, and is better termed the 'medial extension of the trochlear surface'. It is invariably associated with a forward prolongation of the medial articular surface of the talus (Text-fig. 1C).

(c) Rarely, a facet is present on the upper medial surface of the neck which does not follow the line of curvature of the trochlear surface and is separated from this surface by a transverse ridge of bone not covered with articular cartilage. This facet does not articulate with the tibia in dorsiflexion; its exact causation is therefore obscure (Inkster, 1927). However, it appears to be associated with the habit of squatting and it may therefore legitimately be termed the 'medial squatting facet' (Text-fig. 1E).

(d) It is not uncommon for the anterior margin of the trochlear surface of the talus to present a sinuous form due to forward extension of the lateral one-third of this surface. This extension continues the line of curvature of the trochlea and thus makes contact with the undersurface of the tibia during dorsiflexion. It is not a true squatting facet and is therefore better termed the 'lateral extension of the trochlear surface' (Text-fig. 1D).



Text-fig. 1. The various types of talus described in the text. A, normal European talus; B, forward prolongation of the medial articular surface; C, medial extension of the trochlear surface; D, lateral extension of the trochlear surface; E, medial squatting facet (after Inkster); F, lateral squatting facet.

(e) Finally, the facet originally described by Thomson (1889) is a smooth, cartilage-covered area on the upper, lateral surface of the neck of the talus, articulating in full dorsiflexion with a well-marked facet on the anterior surface of the lower extremity of the tibia. It is often divided from the anterior margin of the trochlea by a distinct groove; in other tali it is continuous with the trochlear surface, though always making a sharp angle with the line of curvature of the latter. This may be called the 'lateral squatting facet' (Text-fig. 1F).

#### MATERIAL

One hundred cartilage-covered adult European tali have been studied. These specimens represent approximately seventy dissecting-room subjects, in about thirty of which both tali were examined and in the remainder only one, usually the right. In addition, one talus was examined from each of fifty-six stillborn European fetuses.

## FINDINGS

Forward prolongation of the medial articular surface of the talus upon the talar neck is not uncommon in the adult series (Pl. 1, fig. 1). The prolongation can be expressed numerically by measuring the percentage of this medial surface that lies in front of the anterior margin of the trochlea (Table 1). In the series of foetal tali the medial surface was prolonged anteriorly to a greater extent than in the adult; correspondingly, the posterior end of the medial surface was usually situated well anterior to the posterior margin of the trochlea. It would appear that during growth there is a backward shift of the medial articular surface of the talus relative to the upper surface.

A medial extension of the trochlear surface was present in eleven of the adult tali (Pl. 1, fig. 2). In the foetal series the incidence was much higher—the medial extension of the trochlear surface was definite in forty-four out of fifty-six specimens (Pl. 1, fig. 6). Detailed findings are recorded in Table 2.

No medial squatting facets were present in either the adult or the foetal series. A lateral extension of the trochlear surface was present in seventeen adult tali (Pl. 1, fig. 3). It occurred commonly in the foetal series, but its exact incidence was not recorded. A lateral squatting facet was present in only two adult tali (Pl. 1, figs. 4, 5), but there were thirteen well-marked examples in the foetal series, each associated with a corresponding facet on the tibia (Pl. 1, figs. 6, 7). The incidence in the foetus is detailed in Table 2.

Table 1. *Forward prolongation of the medial articular surface of the talus*

Percentage of medial surface extending beyond front of trochlear surface	Incidence	
	Adult tali	Foetal tali
0-10	48	10
10-20	34	11
20-30	17	24
30-40	1	11

Table 2. *The incidence of two talar facets defined in the text*

Type of talus	Total number	Medial extension of trochlear surface present	Lateral squatting facet present
Foetal European			
5-10 cm., C.R.	11	7	1
10-15 cm., C.R.	28	22	5
15-20 cm., C.R.	11	9	5
20 cm., C.R. and above	6	6	2
Total	56	44	13
Adult European	100	11	2
Adult Panjabi (Charles)	53	Not recorded	34 (+6 doubtful)
Adult Australian (Inkster)	150	Not recorded	45 (+10 doubtful)

## DISCUSSION

It is clear that, relative to the upper surface, the medial articular surface of the talus is situated more anteriorly in the foetus than in the adult. In this respect the European foetal talus resembles the adult talus of other races, such as the Panjabi



(Charles, 1894), the ancient Egyptian (Sewell, 1904) and the Australian (Inkster, 1927).

Medial extension of the trochlear surface is much more common in the European foetus than in the adult (Table 2). Lateral extension of the trochlear surface occurs frequently in the present series but no exact comparison with non-European tali is possible. The medial squatting facet is known to be rare in both European and Australian tali (Wood, 1920); hence the failure to discover an example in the present series.

Finally, the incidence of lateral squatting facets requires especial consideration, since it is this facet which most clearly distinguishes the adult European talus from that of many other races (Table 2). This squatting facet undoubtedly occurs in the Panjabi foetus and infant (Charles, 1894). Unfortunately its exact incidence is not known, but Charles implies that it is common. It is evident from the present findings that it occurs quite often in the European foetus also. Similar facets are not uncommon in animals in which the fully dorsiflexed talus is required to transmit the body weight, as, for example, the tree-kangaroo. Many men of European stock evidently possess these facets at birth, but the lack of subsequent pressure upon them (such as would occur during squatting) allows the attachment of the capsular ligament to encroach upon and obliterate them. Unless the incidence of lateral squatting facets in the Oriental foetus proves to be significantly higher than in the European foetus, it is unnecessary to invoke the hypothesis that acquired characters are inherited in order to explain this difference between the European and Oriental talus.

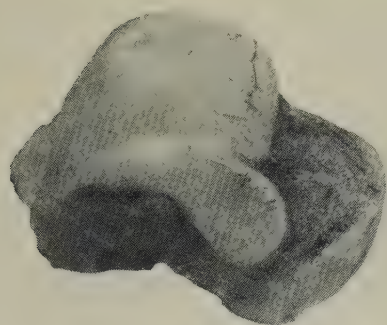
#### SUMMARY

1. The various types of articular facet that may occur on the neck of the human talus are defined.
2. A series of 100 adult and fifty-six foetal European tali have been examined with the object of determining the incidence of squatting facets.
3. The rarity of a lateral squatting facet as described by Thomson (1889) distinguishes the adult European talus from that of many other races. This facet is commonly found in European foetuses, however.
4. The racial differences found in human tali can be explained without necessarily invoking the theory that acquired characters are inherited.

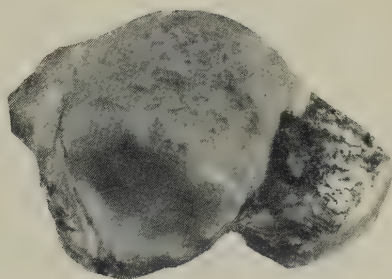
I am indebted to Dr J. R. Napier for his assistance in the collection of adult material, and to Dr R. G. Inkster for placing his own findings at my disposal. Thanks for the provision of material are due to Prof. D. V. Davies, Prof. H. A. Harris and Prof. M. F. Lucas Keene. Mr A. L. Wooding and Mr J. B. Chanter are responsible for the photographic work.

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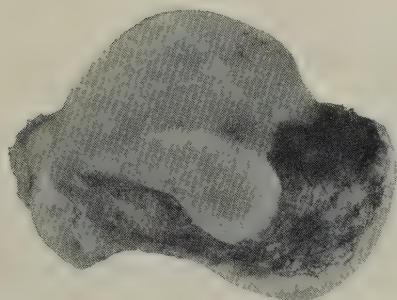
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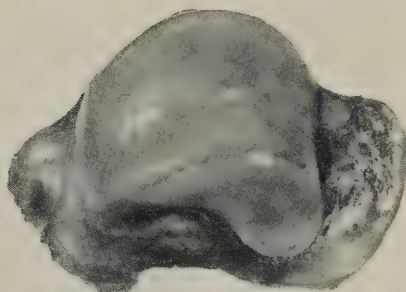
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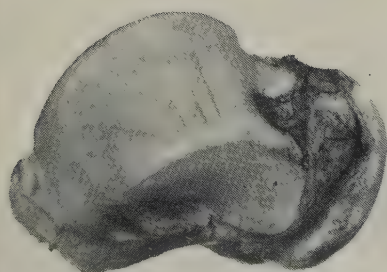
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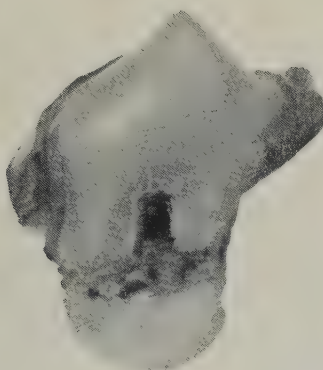
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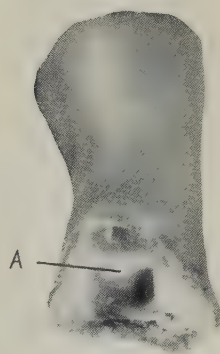
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EXPLANATION OF PLATE

- Fig. 1. Forward prolongation of the medial articular surface.
- Fig. 2. Medial extension of the trochlear surface.
- Fig. 3. Lateral extension of the trochlear surface, associated with some forward prolongation of the medial articular surface.
- Fig. 4. Lateral squatting facet.
- Fig. 5. Lateral squatting facet associated with medial extension of the trochlear surface.
- Fig. 6. Lateral squatting facet and medial extension of the trochlear surface (foetal talus; c.r. length 18 cm.).
- Fig. 7. Left leg of foetus (c.r. length 16 cm.), showing facet on tibia (4).

# TEMPORO-MANDIBULAR MENISCECTOMY IN RABBITS

By R. SPRINZ\*

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Many attempts have been made to bring the function of all intra-articular menisci under one heading (Lotsch, 1928; MacConaill, 1932, 1946; Fairbank, 1948). The proposals put forward have not been generally accepted. Le Gros Clark (1952) summed up the present position when he stated: 'several factors, functional and morphological, are probably concerned with their development, and that from this point of view they have not all an equal significance'.

Regeneration of the menisci after removal has been described in the knee joint. Gibson (1931), Fisher (1936) and Smillie (1943) have given accounts of regeneration of the medial and the lateral meniscus in man. Lukjanov & Pokrovski (1929), Gibson (1931) and King (1936) performed meniscectomies on dogs. Walmsley & Bruce (1937) undertook this operation on rabbits and showed that 22 days after operation the menisci had regenerated. (Some doubt exists as to the amount of cartilage contained in the regenerated structure.)

There is no conclusive evidence that regeneration occurs after removal of the mandibular meniscus. Reports on meniscectomy have been made on man by Pringle (1918), Wakeley (1929), Boman (1947), Dingman & Moorman (1951), Hankey (1953). The literature on experimental work on this joint is sparse. Mathieu (1868) produced artificial displacement of the mandibular menisci on human cadavers; Moose (1941) injected sclerosing solutions into the joints of monkeys. In 1937, Dubecq described experiments on the mandibular joint in rabbits. He excised and traumatized the meniscus of this joint and found that 6 months after excision no regeneration had occurred.

The present investigation was undertaken to determine whether the changes following mandibular meniscectomy in the rabbit were comparable with those observed by Walmsley & Bruce in the knee joint of the same animal.

The answers to the following questions were therefore sought:

- (1) Does the articular disc regenerate after excision?
- (2) Is there any demonstrable change in the efficiency of the chewing mechanism?
- (3) Are there any changes in the joint surfaces?

## MATERIALS AND METHODS

Twenty-seven rabbits were operated upon; two animals did not survive the operation. Eighteen were 6 weeks old, the remaining seven were adult.

The animals were anaesthetized with intravenous Nembutal. A small area between ear and eye was shaved. Under aseptic conditions an incision was made over the jugal bone in the line joining the external auditory meatus to the lateral canthus of the eye. The acute angle between the lateral margin of the bony orbit and the backward prolongation of the zygomatic arch forms an excellent guide to the joint. The deep fascia was incised over the joint, care being taken to keep close

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to the margin of the bony orbit to avoid the posterior facial vein. The capsule of the joint was exposed and opened, the intra-articular disc being identified and grasped with fine forceps. The disc was usually found to be firmly attached to the anterior part of the capsule and was cut away with a fine pointed scalpel (Pl. 1, fig. 1). The capsule and skin were closed with interrupted stitches.

In the control operation the same procedure was adopted, the wound being closed after identification of the meniscus. Recovery was normal in all but the two cases mentioned. Post-operative infection occurred in one case, but the condition subsided on removal of a buried suture.

The animals were kept for 4, 8, 16 and 32 weeks after operation, during which period a note was taken weekly of:

- (a) The weight of the animal.
- (b) Change in size of the persistently growing incisors.
- (c) State of eruption of the small palatally placed incisor.
- (d) State of cleanliness of the teeth.

The animals were sacrificed by bleeding under urethane anaesthesia. The mandible was disarticulated, and the condition of the meniscus examined. The shape and size of the condylar process of the mandible and the articular surface of the temporal bone were noted.

## RESULTS

The results were compared with a normal temporomandibular articulation. Such a joint presents a pear-shaped mandibular condyle, the long axis of which is placed antero-posteriorly (Pl. 1, fig. 3B, D). On the circular part, situated anteriorly, rests the meniscus which articulates with the narrow zygomatic process of the temporal bone above. Peripherally the meniscus is attached to the capsule surrounding the joint. This attachment is stronger anteriorly where the meniscus joins with the lateral pterygoid and masseter muscles.

The meniscus is composed of dense fibrous tissue and contains no cartilage cells. Its vascularity is restricted to a narrow zone in close relation to the capsule; no blood vessels were observed centrally. Both mandibular and temporal articular surfaces are covered with a thin layer of fibro-cartilage.

### (1) *Regeneration of the intra-articular meniscus*

There was no evidence of regeneration in any joint where the meniscus had been wholly or partially removed. All such joints showed condylar enlargements. The condylar processes could be divided into two classes, according to their outline; (i) showing a regular outline with a smooth surface, and (ii) showing an irregular outline with an uneven surface.

In the control operations, the meniscus remained intact and appeared normal with the exception of one animal. In this case (C.S. 10) complete excision was performed on one side and a control operation on the other. There was no regeneration on the operated side, nor was it possible to demonstrate a meniscus on the control side. In the other three cases in which the meniscus was removed on one side and a control operation performed on the other side, the meniscus on the control side remained normal.



(2) *Effects on the efficiency of the joint*

The animals took their food without difficulty. Assessments were made of the masticatory efficiency by following the animals' weight and studying the condition of the teeth weekly.

(a) *Weight*

All 6-week-old animals gained weight at the normal rate. Of the seven adult animals, five gained weight, two lost some weight.

(b) *Change in the persistently growing incisor teeth*

There was no marked change in the eruption or the natural wear of these teeth. In one case (C.S. 9) oblique wearing of the incisor teeth occurred. At autopsy 16 weeks after operation, a greatly enlarged left mandibular condyle was observed which had considerably restricted the movement of the left joint.

(c) *Changes in the palatally placed incisor teeth*

The position of the palatally placed incisors varies in relation to the palate as well as to the persistently growing teeth in individual rabbits. It was therefore not possible to obtain an accurate test of the eruptive states of these teeth. They did, however, seem to over-erupt in those animals that had a unilateral removal of the meniscus. In two such animals the small incisors came to protrude beyond the incisive edge of the persistently growing teeth (Pl. 1, fig. 2).

(d) *State of cleanliness of the teeth*

In the early post-operative period the teeth of all animals became covered with a thick mucous film. The film persisted longer in animals subjected to unilateral than to bilateral meniscectomy.

(3) *Changes in the joint surfaces*

The mandibular condyle enlarged at the same time as the articular surface of the temporal bone became wider. The condylar outline changed from pear-shaped to oval. The articular facet showed either a smooth and regular, or a rough and irregular surface after meniscectomy (Pl. 1, fig. 3). An analysis of the maximum measurements of the length and breadth of operated as compared with normal condyles is given in Table 1. The increases in size of the operated compared with normal condyles is statistically significant.

Table 1. *Analysis of the maximum measurements of length and breadth of operated as compared with normal condyles (measurements in mm.)*

Right side		Left side	
Breadth	Length	Breadth	Length
Normal animals			
4.4 ( $\pm 0.3$ )	10.1 ( $\pm 0.6$ )	4.3 ( $\pm 0.2$ )	9.2 ( $\pm 0.6$ )
Mean of nine observations		Mean of eight observations	
Animals after meniscectomy			
6.2 ( $\pm 0.4$ )	12.7 ( $\pm 0.3$ )	6.2 ( $\pm 0.3$ )	11.9 ( $\pm 0.5$ )
Mean of thirteen observations		Mean of fourteen observations	

(Standard deviation shown in brackets.)

*Histological examination*

The operated joint shows no intra-articular meniscus in any of the serial sections made through the joints. The articular surfaces are covered with greatly thickened fibro-cartilage as compared with the normal joint. These changes do not correspond to a true traumatic arthritis as there was no wearing away of the cartilage surface, and no bone had been laid bare (Pl. 1, figs. 4, 5).

## DISCUSSION

The experimental results give a definite answer to the first question set out in the introduction. The intra-articular meniscus of the mandibular joint does not regenerate after excision in rabbits. This finding is opposite to the observations of Walmsley & Bruce with regard to the menisci of the knee joint of the same animal. The results indicate that there is no foundation for the suggestion that regeneration of the meniscus occurs here as it does in the knee. It confirms the observations of Dubecq and corresponds with the findings of Boman (1950) who, describing the results of a bilateral meniscectomy on a woman who was killed in an accident 9 months after the operation, shows that no regeneration of the menisci occurred. The illustrations in his paper closely resemble the photo-micrograph seen in Pl. 1, fig. 5. Steinhardt (1934) speaks of 'discless joints' in edentulous patients where the menisci were worn away. This evidence suggests that the mandibular meniscus in man has no power of regeneration.

The effect on the masticatory mechanism indicates that unilateral and bilateral meniscectomy is compatible with the healthy growth of the young and the well-being of the adult animal. There is little doubt that animals subjected to a bilateral meniscectomy fared better than those subjected to a unilateral operation. In the latter animals there was some impairment in the masticatory efficiency which led to an over-eruption of the palatally placed incisor teeth, there was also a persistence of a yellow mucous film on all teeth for some weeks after operation. These observations give the answer to the second question set out in the introduction; changes are observable in the efficiency of chewing, but these are slight. The effect of the operation is not derogatory to the well-being of the animal.

The third question referred to the changes on the joint surfaces. These were a definite overgrowth of the condyle after meniscectomy. The degree of this enlargement varied considerably in different cases. There appeared to be no relationship between the amount of overgrowth of the condyle and time after the first 4 weeks. Animals sacrificed at 4 weeks showed the same variation in enlargement as did those sacrificed at 8, 16 and 32 weeks. There was also no marked variation between the size of condyles following unilateral as compared with the size of condyles following bilateral operations. In the rabbit (C.S. 9), already mentioned, the overgrowth was so marked as to restrict greatly the movement on one side. The differences observed in the smoothness of the articular surfaces may have been due to damage to the articular surface at the time of operation, though this factor is difficult to assess. All condyles of animals on whom meniscectomy was performed showed enlargements which more than filled the space left by the removed meniscus. The temporal facet became widened in consequence. Therefore, a joint cavity remained and jaw movement generally was little impaired.

Mandibular meniscectomy may involve removal of more tissue capable of reforming the meniscus than a similar operation on the menisci of the knee joint. There was, however, no regeneration when the mandibular meniscus was only partially excised. The fact, therefore, that the menisci of the knee joint regenerate and the meniscus of the mandibular joint does not, suggests that menisci may meet different functional needs in different joints.

#### SUMMARY

Intra-articular menisci of the mandibular joints were removed in rabbits.

1. Excision of the meniscus does not lead to regeneration in 6-week-old or adult rabbits.

2. Removal of the meniscus did not impair the feeding habits; the post-operative condition was better following bilateral than unilateral operations.

3. Both mandibular and temporal joint surfaces enlarged.

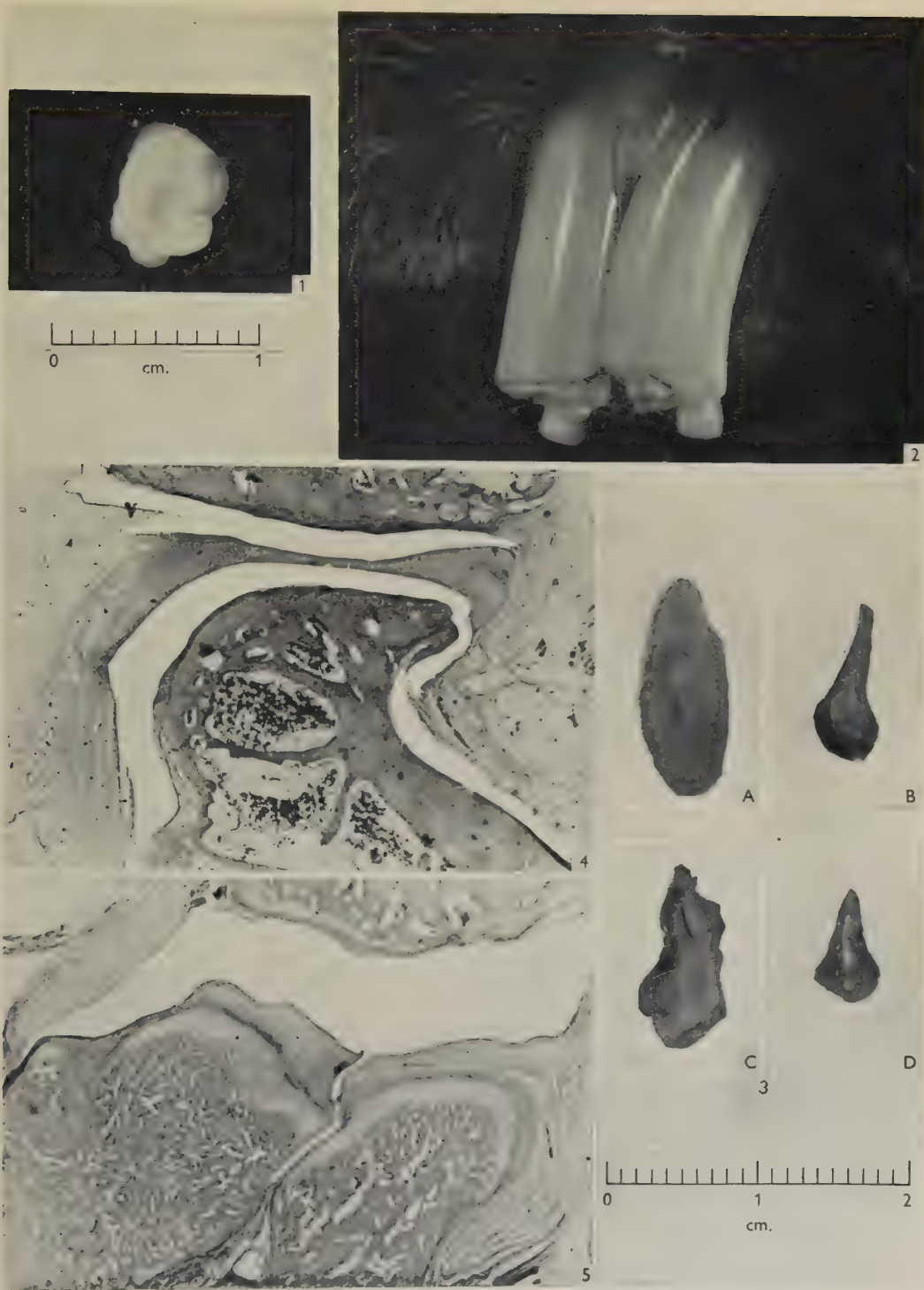
I wish to thank the Nuffield Foundation for affording me the opportunity to undertake this research.

I also wish to thank Prof. Causey, at whose suggestion this work was undertaken. My thanks are also due to Mr Edwards for the illustrations.

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## EXPLANATION OF PLATE

- Fig. 1. Excised meniscus from a 6-week-old animal (C.S. 36).
- Fig. 2. Over-erupted palatal incisor teeth 8 weeks after left menisectomy on an adult animal (C.S. 21).
- Fig. 3. A. Right condyle 4 weeks after right menisectomy, showing a smooth regular articular surface. B. Left condyle, normal (C.S. 13). C, right condyle 4 weeks after menisectomy showing a rough irregular articular surface. D, left condyle, normal (C.S. 34).
- Fig. 4. Transverse section through normal joint showing position of intra-articular meniscus.
- Fig. 5. Transverse section through an operated joint (16 weeks after menisectomy). The articular surfaces are covered with a thick layer of fibro-cartilage, and the meniscus is absent (C.S. 40).



## THE BLOOD SUPPLY OF THE FACIAL NERVE

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The precise aetiology of Bell's palsy, or idiopathic facial paralysis, has not been defined, but the opinion most generally held is that the paralysis is due to ischaemia of the facial nerve trunk. It is therefore surprising to find no general agreement on the gross form of the blood supply to the facial nerve, and no information in the literature on its intrinsic vascular anatomy.

The first account of the blood supply of the nerve is that of Bartholdy (1897), who collated the observations made by earlier authors and built up from them a complete picture of the pattern of arterial supply. As might be expected, this account contained several inconsistencies and items of conflicting evidence. The arterial supply of the terminal branches was investigated by Tobin (1943), and Guerrier (1951) gave a further account of the gross pattern of arterial branches to the nerve which did not agree, in several respects, with the earlier observations quoted by Bartholdy. It therefore seemed necessary to re-investigate the gross features of the blood supply of the facial nerve as well as to study its intrinsic vascular arrangements.

### MATERIAL AND METHODS

Thirty human temporal bones were removed at post-mortem from twenty-seven subjects whose ages ranged from 14 to 85 years: the heads of two full-term foetuses were also available.

The foetal heads were injected, one with neoprene, the other with indian ink in double strength plasma, and the extra-petrous portions of the nerves were dissected in both specimens. One temporal bone was removed from each and sectioned serially.

In eight of the adult specimens the basilar artery was injected, and in fourteen specimens injection was made into the stem vessel giving rise to the stylomastoid artery. In both instances the injection mass used was either 5% indian ink in double-strength plasma, or coloured neoprene. In a further group of eight specimens no injection was made. The facial canal was opened under a binocular microscope and those specimens which had been injected with neoprene were studied to find the gross form of the blood supply to the nerve. Those injected with indian ink in plasma were similarly studied; the nerve was afterwards removed from its canal and either mounted flattened on a slide, or else embedded and sectioned before mounting, in order to show the intrinsic vascular pattern.

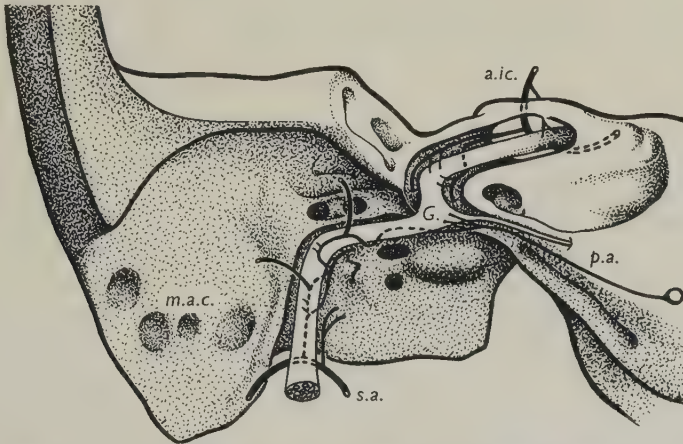
In the uninjected specimens the nerve was stained by the sodium nitroprusside-benzidine technique. Some nerves were mounted flattened out on slides, and others were embedded in gelatin and frozen sections were cut at  $100\mu$  before staining. Details of preparation did not differ from those described by Pickworth (1934) except in regard to the methods of embedding and final mounting. Nerve segments were embedded in gelatin blocks which were left unfixed and unhardened: after

sectioning, the gelatin dissolved away in the warmed staining solutions without adversely affecting the benzidine reaction. Sections mounted in polystyrene mounting medium remained unfaded for 3 months on an average; when neutral balsam was used the stain survived for less than 2 weeks.

### THE GROSS BLOOD SUPPLY

#### *(a) From the pons to the floor of the internal auditory meatus*

Between its exit from the pons and the internal auditory meatus the facial nerve receives branches directly from the anterior inferior cerebellar artery (Pl. 1, fig. 1). This vessel runs laterally from the basilar artery and forms a loop which lies between the facial and auditory nerves and projects towards, and often into, the internal auditory meatus (Stopford, 1916; Nabeya, 1923; Konaschko, 1927). The loop usually gives rise directly to the internal auditory artery but, in one of the eight adult specimens and in one of the foetal temporal bones, the anterior vestibular and vestibulo-cochlear branches took separate origin from the loop of the anterior inferior cerebellar artery. In all of the specimens examined fine vessels passed from both main branches of the internal auditory artery to supply the facial and auditory nerves.



Text-fig. 1. A diagrammatic representation of the right facial canal opened in its whole length. The arteries are shown as solid black lines which are broken where the vessels pass on the medial side of the facial nerve.

#### *(b) In the facial canal*

In its course in the canal the facial nerve receives branches from both the petrosal branch of the middle meningeal artery and the stylomastoid artery (Text-fig. 1).

#### *The petrosal artery*

Immediately after it has entered the skull through the foramen spinosum the middle meningeal artery gives off a short stem vessel which divides into the petrosal artery laterally and a branch to the trigeminal ganglion medially. The petrosal artery is sometimes double and when this is the case the second vessel may arise either from the same common trunk or from the accessory meningeal: each of these

arrangements has been met with twice in a series of eighteen dissections, and in all of the four specimens an anastomosis linked the two vessels. The petrosal artery runs laterally in company with the greater superficial petrosal nerve to enter the temporal bone through the hiatus Fallopii (Pl. 1, fig. 2). It gives off a short, stout branch which divides into a spray of fine vessels to the geniculate ganglion, and is then continued along the facial canal below the horizontal part of the facial nerve to anastomose with the stylomastoid artery. The anastomotic branch gives off a vessel which crosses the lateral side of the facial nerve and ramifies in the roof of the middle ear cavity.

### *The stylomastoid artery*

The stylomastoid artery is usually described as taking direct origin from the posterior auricular artery but that arrangement has not been met with in eighteen dissections in this series. Below the stylomastoid foramen either the posterior auricular artery or the occipital artery gives off a common stem vessel which divides into a number of branches below the base of the skull; these branches pass to the tympanic plate, styloid process and mastoid process and to the post-auricular skin and the facial nerve (Pl. 1, fig. 3). A further branch, which is the stylomastoid artery, enters the stylomastoid foramen on the medial side of the nerve. It was indirectly derived from the occipital artery twelve times and from the posterior auricular artery six times.

The stylomastoid artery forms a loop in the lower part of the facial canal, the returning limb of which leaves the canal with the posterior auricular nerve. From the convexity of the loop two branches usually arise: the largest of these accompanies the facial nerve and the other rapidly breaks up into branches which pierce the posterior meatal wall in company with filaments from the auricular branch of the vagus. The main ascending branch (Pl. 1, fig. 4) lies on the medial side of the facial nerve as far as the junction between its horizontal and vertical parts and then loops around the inferior aspect of the bend, usually passing somewhat on to its lateral side, to reach the infero-medial aspect of the horizontal part of the nerve. It anastomoses directly with the petrosal artery to form a complete arterial arcade in the facial canal.

From the arcade branches are given to the nerve close to the origin of the chorda tympani and at the level of the second bend; a separate branch accompanies the chorda tympani, and one or two branches run through the posterior wall of the canal to supply the mastoid air cells. From the horizontal part of the arcade a vessel leaves the facial canal and ramifies in the roof of the middle ear cavity. The lowest branch from the stylomastoid artery to the facial nerve is therefore at the level of the origin of the chorda tympani.

### *(c) The extra-petrous course*

Below the stylomastoid foramen the nerve is supplied by a constant branch from the stem vessel of the stylomastoid artery (Pl. 1, fig. 3), and, in the parotid gland, it receives twigs from either the posterior auricular artery or the occipital artery, and also from the superficial temporal and transverse facial arteries. The terminal branches of the nerve as they leave the parotid gland are each accompanied by a



fine arteria comitans which gives branches into the nerve and accompanies it until close to its termination.

All the arterial branches to the facial nerve below the stylomastoid foramen are linked longitudinally by free anastomoses in the epineurium.

#### *Venous drainage*

In the internal auditory meatus the nerve is loosely fascicular in structure and contains little connective tissue between its fasciculi; it is widely separated from the periosteum of the meatal walls and its veins drain into branches of the internal auditory vein. At and below the geniculate ganglion the nerve is surrounded by a tough connective tissue sheath which is continuous on the one hand with the periosteum of the facial canal and on the other with the epineurium. The sheath encloses both the facial nerve and the arterial arcade, and in its substance lies a well-marked venous network which drains, anteriorly, into veins accompanying the petrosal artery and, inferiorly, into the venae comitantes of the stem vessel from which the stylomastoid artery is derived. Venous channels in the sheath also communicate with those in the surrounding bone (Pl. 1, fig. 5) and in adult material injected with indian ink venous channels in the walls of the canal were always filled through these connexions. Towards the funnel-shaped opening of the stylomastoid foramen the connective tissue sheath becomes progressively thicker and more dense, and below the stylomastoid foramen it fuses with the carotid sheath on the medial side, and behind, with the dense connective tissue deep to the mastoid process.

#### THE INTRINSIC VASCULAR ARRANGEMENTS

Throughout the facial nerve there is a well-marked intrinsic plexus composed of capillary vessels which run mainly in a longitudinal direction and form a branching network connected at irregular intervals by transverse vessels (Pl. 2, fig. 6).

Just above the bifurcation of the facial nerve in the parotid gland the capillary plexus is more irregular, and in this situation venules are carried into the nerve with the abundant interfascicular connective tissue (Pl. 2, figs. 7, 8). In the horizontal and vertical segments of the nerve vessels of this calibre are for the most part confined to the sheath, and the intrinsic plexus is mainly made up of capillary vessels.

At the geniculate ganglion the density of the capillary bed is much greater than elsewhere in the course of the facial nerve (Pl. 2, fig. 9) and this increased density is constantly shown in both injected and benzidine stained material. The density is greatest at the apex of the geniculate ganglion where most of the ganglion cells are aggregated. The intrinsic vessels of the geniculate ganglion are in continuity with those in the intrinsic plexuses of the nerve. They exhibit many tortuosities, and there are also irregular ampullar dilatations of their lumina which are easily distinguished, in thick sections, from the tortuosities (Pl. 2, figs. 10, 11). They are a striking feature of both injected and benzidine-nitroprusside stained preparations whether sectioned or mounted whole. In structure they are simple endothelial channels with no muscle tissue in their walls. For comparison, the trigeminal and vagal ganglia from four of the same group of subjects have been stained by the sodium nitroprusside-benzidine technique and no obvious ampullar dilatations have been seen.

In its course in the internal auditory meatus the facial nerve has a capillary plexus similar to that seen in the post-genicular parts of the nerve, but rather finer and more open in structure.

#### DISCUSSION

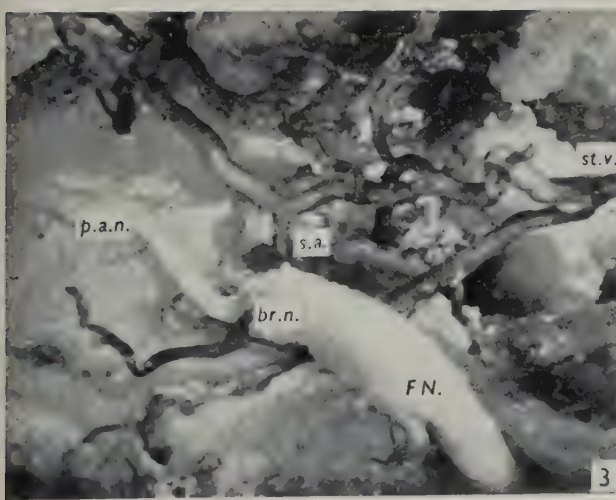
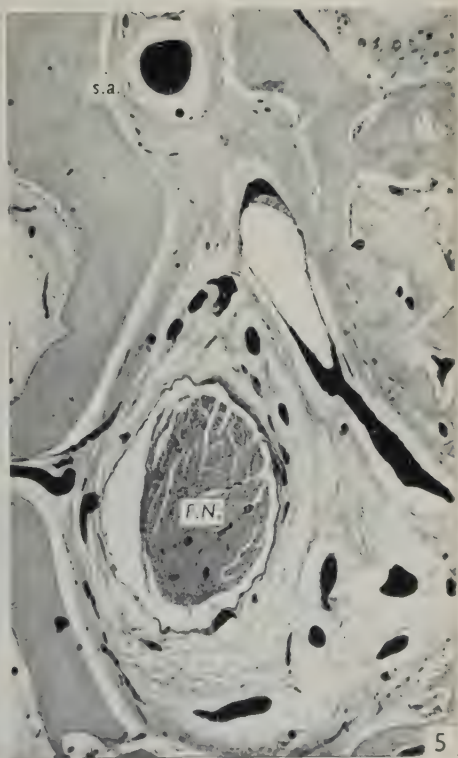
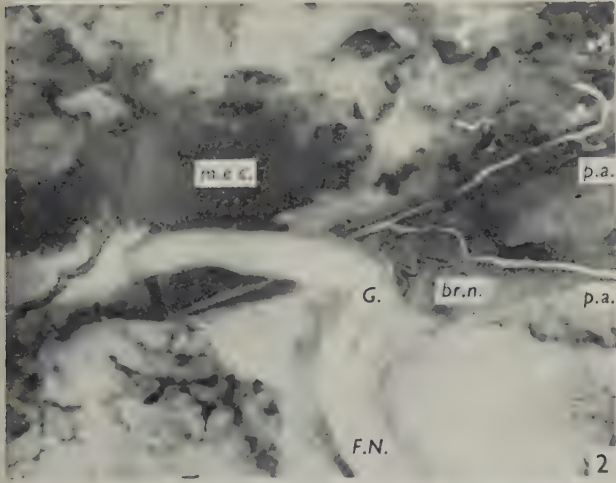
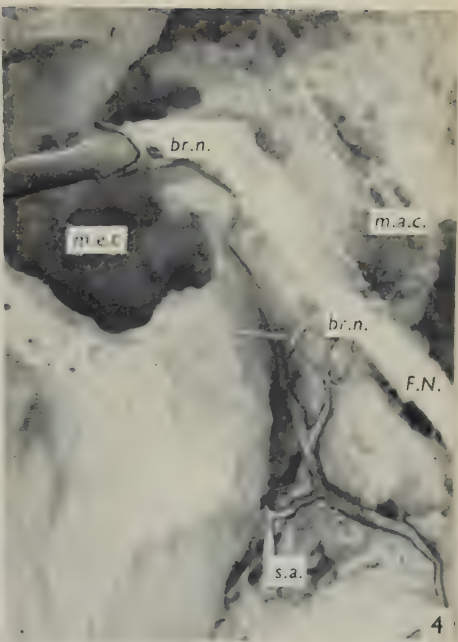
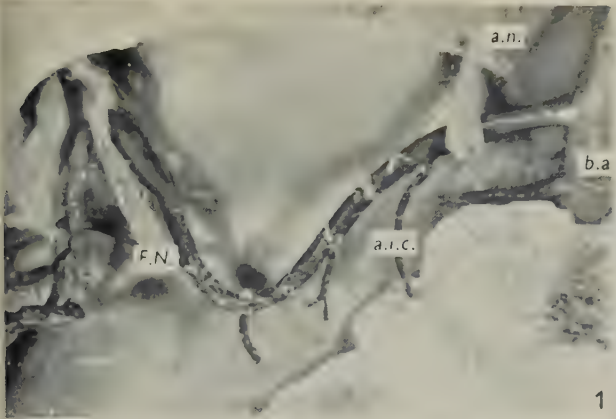
It is evident from the foregoing account that no single vessel exercises a structural autonomy over the vascular arrangements of the facial nerve, either on the arterial or the venous side, although the petrosal artery certainly provides the main arterial supply to the geniculate ganglion. There is no direct link between the petrosal artery and the branches of the internal auditory artery, but, except in this one situation, adjacent arteries of supply are linked to one another either by their parent stem vessels or by free arterial anastomoses. The anastomosis between the petrosal and stylomastoid arteries has been constantly present in this series, although not observed by Guerrier, who does not, however, describe the methods of investigation which he employed: it is therefore assumed that injections were made into some major vessel at a distance from the facial canal. Owing to the well-known difficulty of obtaining full injection of vessels over a diffuse area and especially in bone, it is not difficult to conceive that by such a method the complete length of anastomotic arcade might not become filled.

The channels available for venous drainage from the intra-petrous portion of the nerve are in even freer communication than the arterial branches below the geniculate ganglion, for in this situation there is a marked venous network in the sheath. Moreover, this network communicates with venous channels in the walls of the facial canal.

The irregular capillary and pre-capillary dilatations in the geniculate ganglion are similar to those which have been described in the posterior root ganglia of spinal nerves (Adamkiewicz, 1886; Bergmann & Alexander, 1941). Bergmann (1942) also reports such dilatations in the Gasserian ganglion, but states that they are relatively few in number compared with those in spinal ganglia. It seems probable that they underlie some local feature of the vascular dynamics in both geniculate and posterior root ganglia, and it may be noteworthy that in both these situations the ganglia are protected by the walls of a bony canal. It is also of interest that they are present on the course of the facial nerve in the only situation where it has not been possible to demonstrate direct links between adjacent arteries of supply to the nerve. A similar state of affairs exists in spinal ganglia, for Adamkiewicz stated that their arteries of supply entered from the medial side with the nerve root, and Bergmann and Alexander confirmed this and made the further observation that the anastomosis between the ganglionic vessels and those in the spinal nerve distal to the ganglion was very poor.

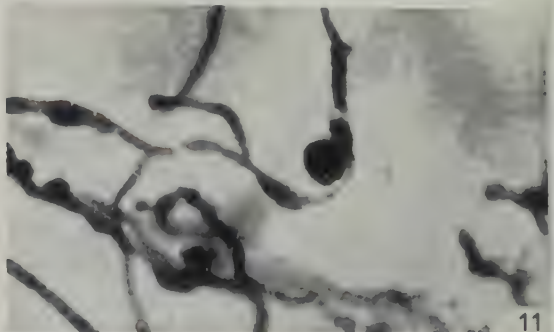
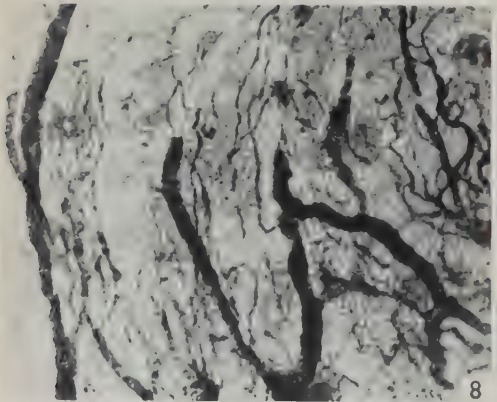
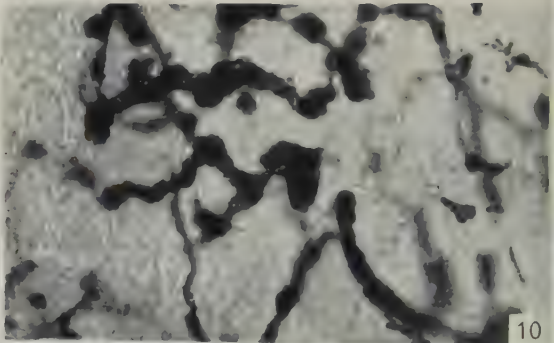
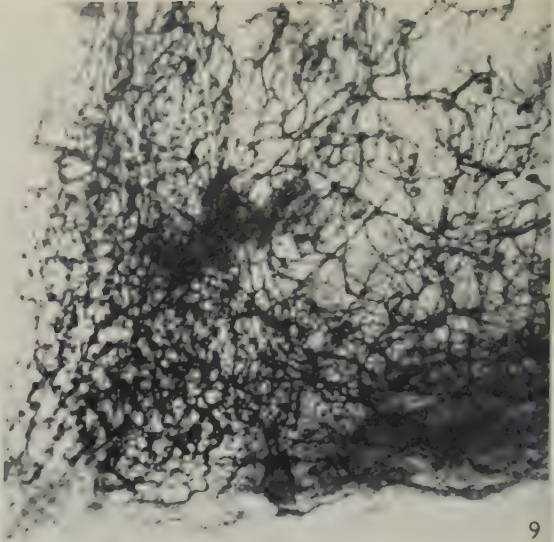
The intrinsic plexus below the geniculate ganglion resembles that seen in peripheral nerves in the limbs. An additional feature, which has not been noted elsewhere, is the unusual disposition of venules in the nerve trunk above its bifurcation.

In conclusion, there is no anatomical evidence of precarious blood supply to any part of the facial nerve below the geniculate ganglion which would especially predispose to ischemia as a result of vascular spasm. It would seem likely that the



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peculiar anatomical situation of the facial nerve is a more potent factor in the aetiology of idiopathic facial paralysis than are the local vascular arrangements.

### SUMMARY

The gross blood supply and intrinsic vascular anatomy of the facial nerve have been described and the findings are discussed. The significance of pre-capillary and capillary dilatations in the geniculate ganglion is also considered.

The author is grateful to Miss D. J. Collier, through whom a grant was obtained from the Royal Free Hospital Endowment Fund, for suggesting the subject of this investigation. Thanks are extended, also, to Prof. R. E. M. Bowden for her very valuable advice and encouragement.

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### EXPLANATION OF PLATES

#### Key to abbreviations used in text-figure and in Plates 1 and 2

<i>a.i.c.</i> anterior inferior cerebellar artery	<i>m.a.c.</i> mastoid air cells
<i>a.n.</i> abducent nerve	<i>m.e.c.</i> middle ear cavity
<i>b.a.</i> basilar artery	<i>p.a.n.</i> posterior auricular nerve
<i>br.n.</i> branch to facial nerve	<i>p.a.</i> petrosal artery
<i>F.N.</i> facial nerve	<i>s.a.</i> stylomastoid artery
<i>G.</i> geniculate ganglion	<i>st.v.</i> stem vessel of the stylomastoid artery

### PLATE 1

- Fig. 1. Full-term foetus, right side: showing the origin of the internal auditory artery from the anterior inferior cerebellar artery. Neoprene injection.  $\times 6$ .  
 Fig. 2. Left temporal bone dissected from above. There is duplication of the petrosal artery: its anastomotic branch and its branch to the geniculate ganglion are shown. Neoprene injection.  $\times 6$ .  
 Fig. 3. Left temporal bone seen from below, showing the stylomastoid artery, its stem of origin, and the artery to the facial nerve below the stylomastoid foramen. Neoprene injection.  $\times 6$ .  
 Fig. 4. The lower dissection of the specimen from which Fig. 2 was photographed. The facial canal has been opened by a transmastoidal approach and the nerve displaced posteriorly. The stylomastoid artery is shown: its main ascending branch anastomoses with the petrosal artery, and branches to the facial nerve are shown. Neoprene injection.  $\times 6$ .  
 Fig. 5. A transverse section through the left temporal bone of a full-term foetus. The facial nerve sheath and its contained venous plexus is shown. Indian ink injection.  $\times 30$ .

## PLATE 2

- Fig. 6. The interfascicular plexus in the vertical part of the facial nerve. Sodium nitroprusside-benzidine stain.  $\times 50$ .
- Fig. 7. The interfascicular plexus above the bifurcation of the nerve. Sodium nitroprusside-benzidine stain.  $\times 50$ .
- Fig. 8. Transverse section of the facial nerve above the bifurcation. Sodium nitroprusside-benzidine stain.  $\times 100$ .
- Fig. 9. Genuiculate ganglion. Flattened preparation. Sodium nitroprusside-benzidine stain.  $\times 30$ .
- Fig. 10. A horizontal section through the apex of the genuiculate ganglion. Sodium nitroprusside-benzidine stain.  $\times 200$ .
- Fig. 11. A horizontal section through the angle of the genuiculate ganglion. Sodium nitroprusside-benzidine stain.  $\times 200$ .



# THE RATE OF RENEWAL OF INTESTINAL EPITHELIUM IN THE CAT

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## INTRODUCTION

The fact that the epithelium of the intestine possesses considerable powers of regeneration has been known for many years, and current text-books of histology draw attention to the numerous mitotic figures that can be seen in the intestinal glands (crypts of Lieberkühn). Pathological lesions of the stomach and intestine involving loss or destruction of epithelium usually show evidence of epithelial repair to a greater or lesser extent, and in many instances complete epithelialization of the lesion occurs. In the case of the small intestine the cells of the crypts are considered to be the source from which replacements are readily available. While an extensive literature exists on the subject of epithelialization following pathological or artificial lesions, some authors (e.g. Ivy, Grossman & Bachrach, 1952) have pointed out that little attention has been paid to the epithelial activity which occurs under physiological conditions. Tissues such as epidermis and the epithelial lining of the alimentary tract are subject to varying degrees of frictional loss of cells, which in a healthy animal is made good by activity in the parent tissue. Using colchicine as a mitotic inhibitor, Leblond & Stevens (1948) made observations on the rate of renewal of intestinal epithelium in the albino rat, calculating that the epithelial cells of the duodenum were replaced every 1.57 days and those of the ileum every 1.35 days. In view of the widespread use of the cat in biological research it was thought desirable to carry out experiments on these animals using colchicine, with a view to estimating the rate of renewal of the epithelium covering the villi.

## MATERIALS AND METHODS

Observations were made on the mitotic activity in the epithelium of the small intestine of adult cats by comparing the number of mitoses seen at any one time in the normal animal with the number seen 5 hr. after the injection of colchicine. With these data, the average duration of a mitotic cycle and the time taken for renewal of the epithelial covering of the villi can be calculated.

All cats of both the normal and colchicine-treated series had been starved since the previous evening. Those used as normal controls were killed at 3 p.m. and portions of the duodenum and of the ileum (40–50 cm. proximal to the ileo-colic junction) were removed and fixed in 80% alcohol. After embedding in paraffin wax, sections were cut at a thickness of  $7\mu$  and stained with haematoxylin and eosin.

To another series of cats intra-peritoneal injections of colchicine were given at 10 a.m. and the animals were sacrificed at 3 p.m. without having been fed during the intervening 5 hr. The colchicine solution was prepared by dissolving the powder

in distilled water or normal saline; stored in a refrigerator it will maintain its potency for many weeks. The dose of colchicine used was 0.25 mg./kg. of body weight, a dose which had previously been found sufficient to arrest dividing nuclei in metaphase (McMinn & Mitchell, 1954). Portions of duodenum and of ileum were removed and fixed as for the normal control animals.

Counts of mitotic epithelial nuclei in sections from both normal and colchicine-treated animals were carried out using a square mask in one of the oculars of a binocular microscope. Contiguous fields were counted from the bases of crypts to the tips of villi, at least 2000 nuclei being included in any single count. Random fields were chosen and counts were made only where the crypts and villi were sectioned approximately throughout their length. Although mitosis normally occurs only in the cells lining the crypts, the number of dividing nuclei is expressed as a percentage of the total number of epithelial nuclei in both crypts and villi. The estimation of nuclear populations from tissue sections is open to a number of errors, and the precautions mentioned by Leblond & Stevens (1948) and by Stevens & Leblond (1953) were observed in the present study.

In normal animals, the identification of metaphase, anaphase and telophase stages of the mitotic cycle was comparatively easy, but to decide when a given nucleus had entered prophase was often difficult. In the present study, nuclei were considered to have entered prophase only when the chromatin material was aggregated into rounded clumps with a tendency to become arranged round the periphery of the nucleus, inside an intact nuclear membrane. Nuclei which may have been in earlier stages of prophase have been included among the resting nuclei, and it is therefore possible that the absolute number of cells in division is somewhat greater than the figures suggest.

Five hours after colchicine injection, arrested metaphases appeared as dense clumps of chromatin in cells which were paler than normal when stained with haematoxylin and eosin, and later phases of the mitotic cycle were not seen.

## RESULTS AND DISCUSSION

The results of the mitotic counts are summarized in Tables 1-4.

To compensate for the constant mitotic activity in the crypts, there must be an equivalent loss of cells if the epithelium is to be maintained in a steady state, and from their observations on the rat, Leblond & Stevens (1948) suggested that cells passed upwards from the crypts along the sides of the villi, from the tips of which they were eventually shed. Subsequent observations with radio-active material (Leblond, Stevens & Bogoroch, 1948) supported this contention. In the present studies it was found that in the cat, as Leblond & Stevens had found in the rat, arrested mitoses following the use of colchicine were often present just above the mouths of the crypts, suggesting that, as mitosis does not normally occur outside the crypts, the cells exhibiting these arrested mitoses had been pushed upwards from below. Also, at the tips of many of the villi, both in the duodenum (Pl. 1, fig. 1) and in the remainder of the small intestine (Pl. 1, fig. 2), 'extrusion zones' could be seen, where distorted cells and nuclei were apparently becoming ejected into the lumen of the gut. Under normal conditions, freshly shed epithelial cells

were not seen in the lumen of crypts, and it would appear that cell loss does normally only occur from the tips of villi.

In the ileum of the normal animal (Table 1), an average of 0.95% of the total number of epithelial nuclei were in mitosis at any one time; the average number of cells seen in arrested mitosis 5 hr. after the administration of colchicine was 3.77%

Table 1. *Percentage of dividing nuclei in ileal epithelium in the normal animal*

	Total no. of nuclei	No. of nuclei in mitosis	Percentage of nuclei in mitosis	Average percentage
Cat 20	2158	20	0.93	1.04
	2584	28	1.08	
	2166	21	0.97	
	2149	25	1.16	
Cat 25	2022	19	0.94	0.80
	2133	16	0.75	
	2050	17	0.83	
	2246	15	0.67	
Cat 28	2109	26	1.23	0.94
	2159	18	0.83	
	2162	18	0.83	
	2172	19	0.88	
Cat 9	2130	29	1.36	1.00
	2158	22	1.02	
	2191	18	0.82	
	2288	18	0.79	

Mean of average percentage: 0.95.

Table 2. *Percentage of dividing nuclei in ileal epithelium 5 hr. after injection of colchicine*

	Total no. of nuclei	No. of nuclei in arrested mitosis	Percentage of nuclei in arrested mitosis	Average percentage
Cat 36	2220	74	3.33	3.74
	2612	108	4.13	
	2425	81	3.45	
	2510	98	4.06	
Cat 31	2293	85	3.85	3.58
	2223	84	3.95	
	2254	75	3.33	
	2190	70	3.19	
Cat 50	2177	84	3.81	3.78
	2317	85	3.65	
	2224	84	3.95	
	2337	87	3.72	
Cat 39	2298	92	4.00	3.99
	2375	95	4.00	
	2377	102	4.29	
	2101	77	3.67	

Mean of average percentage: 3.77.

(Table 2). From these figures it is seen that the number of mitotic nuclei in colchicine-treated animals was approximately 4 times the number seen in the normal animal, so that on the average there were four consecutive mitotic cycles taking place during the 5 hr. of colchicine treatment. This indicated that the average duration of any one mitotic cycle was 1 hr. 15 min., which may be compared with 1 hr. 8 min. in the rat as determined by Leblond & Stevens (1948). In the cat's



duodenum the mitotic duration, as calculated from Tables 3 and 4, is found to be approximately the same as in the ileum.

Mitosis is normally confined to crypt cells, and in the region of the ileum used for the present study approximately 50 % of the total number of epithelial cells was found to line the crypts, the remainder covering villi. Since 3.77 % of the *total*

Table 3. *Percentage of dividing nuclei in duodenal epithelium in the normal animal*

	Total no. of nuclei	No. of nuclei in mitosis	Percentage of nuclei in mitosis	Average percentage
Cat 44	2056	18	0.87	0.88
	2079	17	0.82	
	2122	20	0.94	
	2112	19	0.90	
Cat 24	2088	21	1.00	0.95
	2131	22	1.03	
	2170	20	0.92	
	2229	19	0.85	
Cat 38	2118	18	0.85	0.73
	2051	13	0.63	
	2034	13	0.64	
	2049	16	0.78	
Cat 64	2171	20	0.92	0.94
	2123	20	0.94	
	2090	22	1.05	
	2202	19	0.86	

Mean of average percentage: 0.88.

Table 4. *Percentage of dividing nuclei in duodenal epithelium 5 hr. after injection of colchicine*

	Total no. of nuclei	No. of nuclei in arrested mitosis	Percentage of nuclei in arrested mitosis	Average percentage
Cat 67	2099	83	3.95	3.81
	2325	82	3.52	
	2114	84	3.98	
	2165	82	3.79	
Cat 68	2151	75	3.49	3.26
	2147	66	3.07	
	2169	65	2.99	
	2087	73	3.49	
Cat 3	2199	81	3.68	3.84
	2172	85	3.92	
	2116	85	4.02	
	2119	79	3.73	
Cat 4	2179	87	3.99	3.73
	2099	81	3.86	
	2119	77	3.40	
	2270	81	3.56	

Mean of average percentage: 3.66.

number of cells were in arrested mitosis 5 hr. after colchicine administration, it follows that twice this number (i.e. 7.54 %) of *crypt* cells were mitotic during 5 hr., and that all the crypt cells should have undergone mitosis in 66.2 hr. In this time, therefore, the number of crypt cells should have doubled; but since the total number of epithelial cells remains constant, an equivalent number of cells must pass upwards

to take part in the epithelial covering of the villi, and an equivalent number be shed from the tips of the villi. Since the number of crypt cells and villous cells is approximately equal, the time of 66.2 hr. is the time taken for complete renewal of the villous epithelium. Alternatively, this may be expressed as the time taken by an average cell to pass from the base of a villus to its tip.

The villous cells do not undergo mitosis; hence they can be compared with a column of cells which is continually receiving new cells at the base and shedding old cells at the top. In the ileum this column, representing 50 % of the total number of epithelial cells, is renewed in approximately 2.75 days. The remaining 50 % of cells are crypt cells which are undergoing mitosis; these cannot be compared with a column which is merely receiving cells at its base and shedding them from the top. The crypt cells can be regarded as a self-replenishing reservoir which is constantly delivering cells to the villous group.

In the duodenum, approximately 60 % of the total number of epithelial cells were found in the crypts and 40 % over the villi. From Table 4 it can be calculated that 6.1 % of crypt cells were mitotic during 5 hr. and that all the crypt cells should have undergone mitosis in 82.0 hr. Hence the time required for renewal of the 40 % which covered the villi is 54.6 hr. or approximately 2.25 days.

It is of interest now to compare this calculated rate of renewal with the rate at which epithelium appears to advance over an artificial defect or ulcer. In a recent report on the healing of mucosal lesions in the ileum of the cat by McMinn & Mitchell (1954), mitotic counts, carried out at the margins of 'excision' ulcers of 24 hr. or longer duration in colchicine-treated animals, suggested that the presence of a break in continuity of the epithelium did not serve as an added stimulus to the cell multiplication that was already in progress. It may be inferred from this that epithelium grows over the floor of a defect at a rate similar to that at which it normally passes along the sides of the villi. Observations made from this ulcer material indicated that, by 7 days after the production of an artificial ulcer, epithelium had advanced for a distance of just over  $1000\mu$  from the estimated original margin of the defect. Calculations in the present work show that in 2.75 days epithelium had progressed approximately  $450\mu$ , this being the average distance from the bases of villi to the tips of villi in that part of the ileum which was used for all the experiments, both of the ulcer series and of the present work. In 7 days, therefore, it is calculated to have progressed for just over  $1100\mu$ , a figure which may be compared with  $1000\mu$  in the ulcer material. Clearly no rigid conclusions can be drawn from these observations in view of the difficulty in obtaining precise measurements of the spread of epithelium across a defect whose margins become increasingly difficult to define as healing proceeds. Although in some cases epithelium had progressed as much as  $500\mu$  in 2 days, the cells in this early stage were much flattened compared with the normal columnar form. After the seventh day, the deepening depressions which occurred in the hitherto level epithelial covering and which eventually resulted in the appearance of a typical mucosal architecture with new crypts and villi, rendered accurate measurements even more difficult. Nevertheless, the close coincidence of the figures ( $1000\mu$  compared with  $1100\mu$ ) makes it tempting to suggest that the similarity of the rates of epithelial movement under these varying conditions may be significant.

## SUMMARY

1. With the use of colchicine, the average duration of mitosis among epithelial cells in the small intestine of the cat is found to be approximately 1 hr. 15 min.
2. The time required for renewal of the epithelium covering the villi is found to be approximately 2.25 days in the duodenum and 2.75 days in the ileum.
3. This calculated rate of renewal under physiological conditions is compared with the rate at which epithelium grows over the floor of a small artificial defect in the ileal mucosa; the findings suggest that, for any period longer than 24 hr. after the creation of such a lesion, the rates are similar.

I wish to thank Prof. Francis Davies for his interest and helpful criticism in the preparation of this paper. I am indebted to Mr J. H. Kugler and Mr J. Morrill for technical assistance and the preparation of photomicrographs. Part of the expenses of this work was defrayed by a grant from the University of Sheffield Medical Research Fund, for which I also wish to express my thanks.

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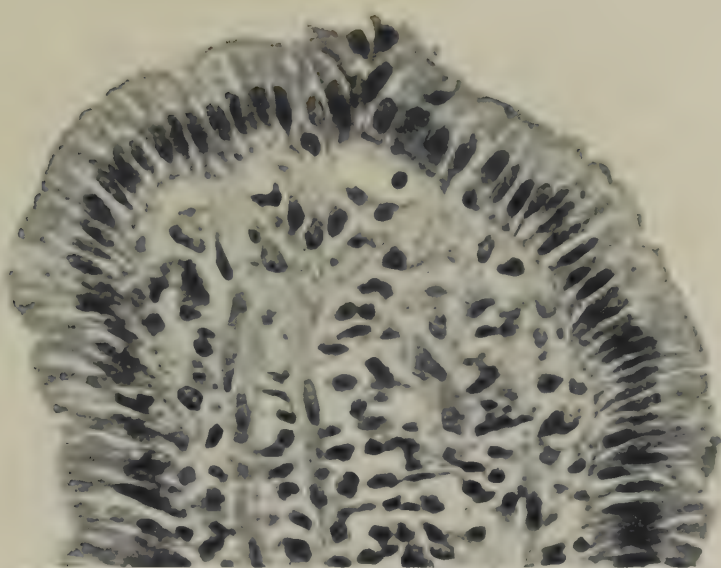
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## EXPLANATION OF PLATE 1

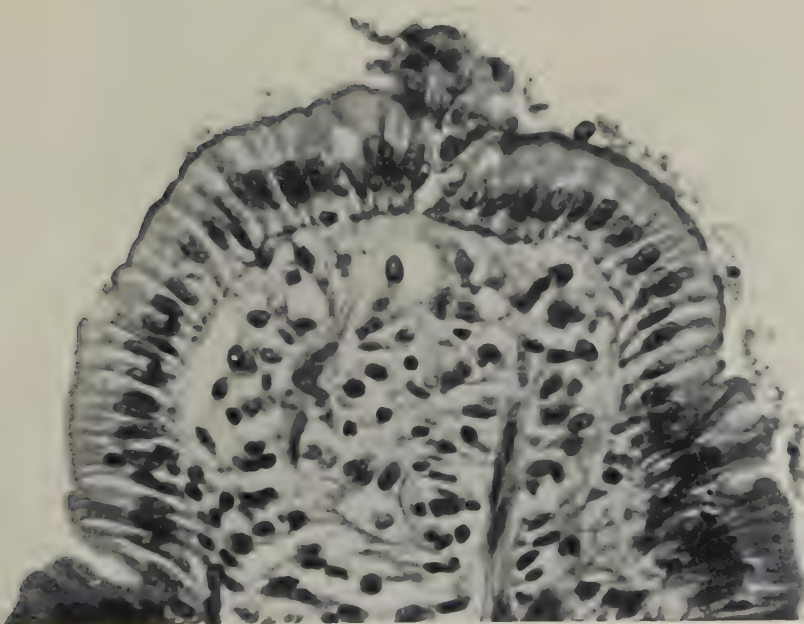
Both sections were stained with haematoxylin and eosin. Magnification,  $\times 520$ .

- Fig. 1. Duodenum. Tip of a villus showing an accumulation of epithelial cells—an early 'extrusion zone'.
- Fig. 2. Ileum. Tip of a villus showing ejection of cells at an 'extrusion zone'. The basement membrane remains intact.





1



2



## TIME OF APPEARANCE OF THE CENTRES OF OSSIFICATION OF THE FIBULAR EPIPHYSES

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### INTRODUCTION

The centre of ossification of the upper end of the human fibula appears at the age of 3-4 years, about 2 years later than that of the lower end. The fusion of the proximal end takes place about the age of 18-20, about 2 years after fusion of the lower epiphysis. In this respect its ossification is different from that of other human long bones, in which the centre of ossification of the epiphysis at the growing end, that is the end which fuses last, appears earlier than the centre of the opposite end.

This difference in ossification of the fibula has been interpreted in various ways. Walmsley (1918) stated that it was the upper end epiphysis which was unusual in that its fusion was delayed and that this was probably due to the fact that it was bound by articulation to the upper end of the tibia at which growth was more active. He also maintained that the upper epiphysis was a traction epiphysis and the times of appearance and fusion of the centres were delayed by the action of the muscle attachments. Wood Jones (1949) connected the growth of the lower end of the fibula during the later months of intra-uterine and immediate post-natal life with the early appearance of its centre of ossification as compared with the proximal. Le Gros Clark (1952) suggested that the times of appearance and fusion of the centre for the lower end of the fibula were precocious and might be related to the strain which it had to withstand when the infant began to walk.

If any or all of these interpretations are correct it must be accepted that mechanical factors play a part in the determination of the times of appearance and fusion of the centres of ossification for the fibula. Yet it is well known that bones become differentiated in explanted tissues entirely removed from their normal environment. Evidence from this field suggests that the pattern of skeletal development is determined in the embryonic mesoderm long before mechanical forces have any effect. If mechanical factors are an important influence the wide differences in form and function of the fibula in different species can be expected to lead to variations in the pattern of its ossification.

The object of this investigation was to determine whether the unusual pattern of ossification of the human fibula was repeated in other species. Since it was assumed that the unusual feature was that the centre of ossification of the upper end epiphysis appeared late, the times of appearance of the epiphyseal centres of ossification of the fibula were investigated. It was thought that the ossification of a fibula articulating with the femur would be of particular interest.



## MATERIAL AND OBSERVATIONS

The times of appearance of the centres of ossification of the epiphyses of the fibula were investigated in the rabbit, guinea pig and cat by means of serial X-ray films and in pigs of different ages and in the kangaroo by X-ray films of two preserved specimens.

*Rabbit.* Eleven animals were investigated. In the rabbit the fibula shows a reduction of its lower end. The upper end articulates with the inferior surface of a lateral projection of the lateral tibial condyle, while the lower end of the shaft fuses with the tibia about halfway along the length of the latter.

At birth the lower end of the fibular shaft is already fused with the tibia and no fibular epiphyseal centres are present. The ossification centre of the upper tibial epiphysis is present.

During the next 10 days two centres of ossification, a medial and a lateral, appear in the lower tibial epiphysis (Pl. 1, fig. 1). On morphological grounds it can be assumed that the lateral centre represents that of the distal end of the fibula which has now become incorporated in the tibia. The two centres enlarge rapidly and by 3 weeks show evidence of fusion. At 5 weeks they form a bilobed mass which fuses with the diaphysis between the 19th and 24th weeks.

The centre for the upper end of the fibula appears during the 3rd week. Fusion with the diaphysis is later than at the lower end and occurs after the 30th week.

*Guinea-pig.* Six animals were investigated. In the adult guinea-pig the upper end of the fibula is fused with the tibia. There is no reduction, the shaft and the lower end being quite separate from the tibia.

At birth, the fibular shaft is well developed and the centre for the distal epiphysis is present (Pl. 1, fig. 2). Fusion of the distal epiphysis with the shaft occurs at about the 25th week.

The centre for the upper end appears about 10 days after birth. Fusion of this end with the upper tibial epiphysis occurs rapidly during the next few weeks, but fusion of the upper epiphysis with the shaft of the fibula is long delayed. It was found that the lower epiphysis had fused by 40 weeks and the upper epiphysis was still separate after 52 weeks. This is confirmed by Zuck (1938) who recorded that the fusion of the lower epiphysis of the fibula occurred by 41–44 weeks but the upper epiphysis was still separate after 2½ years.

*Cat.* Three animals were X-rayed. The fibula shows no reduction.

The shaft is well developed at birth. The centre for the distal end appears at 3 weeks (Pl. 1, fig. 4) and the centre for the proximal end at 6 weeks. Fusion times were not investigated.

*Kangaroo.* In the kangaroo the lateral aspect of the upper articular surface of the fibula articulates with a disc of cartilage which articulates with the femur. Two preserved specimens of unknown age were X-rayed. The fibula showed no reduction. In each case the centre for the distal epiphysis was present, while no centre for the proximal epiphysis could be seen (Pl. 1, fig. 3).

*Pig.* The fibula is complete in the pig, articulating above with the tibial condyle and below or distally with the tibia and tibial tarsal bone medially and the fibular

tarsal bone laterally. The centre for the lower end appears before 2 weeks (Pl. 1, fig. 5) and for the upper end at 4 weeks. Fusion times are given by Payton (1933) as 650 days after birth for the distal end and 730 days for the proximal end.

#### DISCUSSION

Information regarding the ossification of the fibula in the rat, horse and ass, ox and rhesus monkey is available. Dawson (1925, 1934), Strong (1925) and Spark & Alden (1928) investigated the ossification of the albino rat skeleton. From their work it appears that the centre for the fibular shaft is present at 17 days of intra-uterine life, and at birth the lower end of the fibula is in contact with the tibia. Two weeks after birth the bones begin to fuse in their lower thirds. The centre for the distal fibular epiphysis appears 14 days after birth, and that for the proximal epiphysis 28 days after birth.

Fusion of many epiphyses is absent even in elderly rats. It is recorded that fusion of the distal epiphysis of the fibula occurs at 98–130 days, but the proximal epiphysis is still separate at 1135 days.

Sisson & Grossman (1953) state that in the horse the fibula is present in the upper half of the leg. Its head articulates with the lateral condyle of the tibia and it ends below in a pointed extremity attached to a fibrous band. The lateral malleolus of the tibia is really the distal end of the fibula. In the embryo the fibula is represented by cartilage along its whole length, but only the proximal half and extreme distal end ossify. Küpfer (1931) investigated the ossification of the skeleton of the horse and ass but there is some confusion with regard to his observations on the ossification of the fibula. A careful examination of his figures and X-ray reproductions, however, leaves little doubt that the fibula ossifies from three centres, one for the diaphysis and one for each end, and that the ossification of the lower end epiphysis, that is the lateral malleolus of the tibia, begins before that of the upper end.

Sisson & Grossman (1953) describe the fibula in the ox as consisting of an upper end, which is fused with the tibia, and a lower end, which forms the lateral malleolus, remains separate from the tibia and articulates with the tibia and the tibial and fibular tarsal bones. Passantino (1937) investigated the ossification of the fibula in the species *Bos taurus* and in eleven young animals found that the fibula had a distal but no proximal epiphysis.

Although not specifically mentioning the ossification of the fibula, Schultz (1937) reproduced a tracing of the X-ray film of the lower extremity of a rhesus monkey, whose age was 169 conception days. In that tracing are labelled both the proximal and distal epiphyses of the tibia and only the distal epiphysis of the fibula, thus indicating that the distal fibular epiphysis ossifies before the proximal.

These observations together with those of the present investigation show that, despite the diversity of the form and hence the function of the fibula, its pattern of ossification is similar in all the animals which were investigated—the centre of ossification for the distal end always appears earlier than the centre for the proximal end. This is a widespread mammalian characteristic and the ossification of the human fibula cannot be related to any special features of the human skeleton. The peculiar ossification of the fibula can be given as an example of how the basic growth pattern

of a bone is not altered even by very marked differences in form and function, but it is impossible to suggest what it is that determines the unusual pattern of ossification of this bone.

#### SUMMARY

1. As compared with other long bones, the human fibula is peculiar in that the centre of ossification for the proximal epiphysis (the growing end) appears after the centre of ossification for the distal.

2. This peculiarity is found of fibulae in many different types.

3. It is suggested that the pattern of ossification of the human fibula is a widespread mammalian characteristic and is not related to any special features of the human skeleton.

We wish to thank Prof. F. Wood Jones, Dr G. R. de Beer, Dr F. C. Fraser, Dr W. C. Osman Hill and Dr A. A. Vickers for help in preparing this paper, and the staff of the Department of Medical Illustration, Guy's Hospital, for the reproductions of the X-ray films.

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#### EXPLANATION OF PLATE

(X-ray films of legs of several animals showing ossification centre for distal fibular epiphysis and no centre for proximal.)

Fig. 1. Rabbit, 10 days old. (Two centres of ossification for distal end of tibia have appeared, lateral one of which is regarded as that of the distal end of the fibula.)

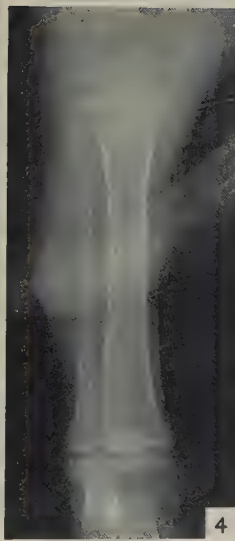
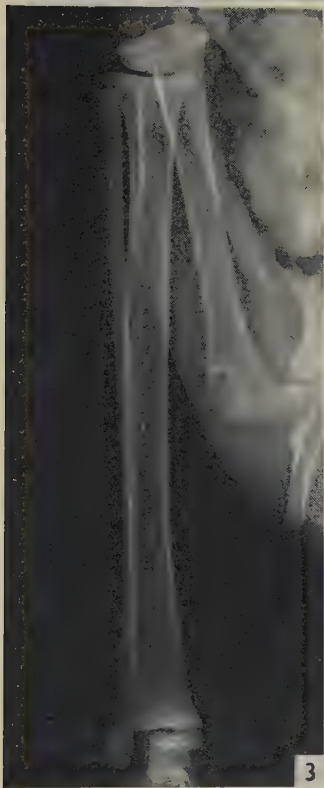
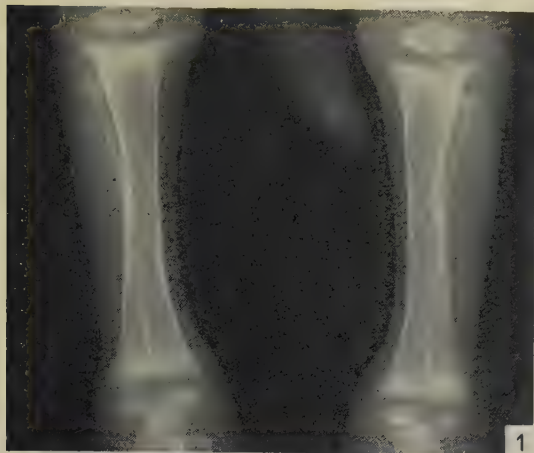
Fig. 2. Guinea-pig, 1 day old.

Fig. 3. Kangaroo, unknown age.

Fig. 4. Cat, 3 weeks old.

Fig. 5. Pig, 2 weeks old.







# THE TERMINAL PHALANX OF THE GREAT TOE

By J. L. WILKINSON

*Anatomy Department, Manchester University*

Studies on hallux valgus commonly contain reference to an outward deflexion of the terminal phalanx at the interphalangeal joint (Emslie, 1939; Hardy & Clapham, 1952). Personal investigations have shown that the apparent deformity in this region lies mainly in the terminal phalanx itself, and that it is a *normal* feature of the human foot.

## MATERIAL AND METHODS

The feet of thirty medical students (thirteen male, seventeen female; aged 18–21 years) and the terminal phalanges from the great toes of thirty-five aged cadavers have been examined radiologically. Measurements of the degree of diaphysial angulation are necessarily approximate, but by taking a series of mid-points distal to the epiphysis a line may be constructed which represents with moderate accuracy the plane of the shaft. Seventeen great toes from adults and ten from infants have been dissected. A great toe from each of three male and three female foetuses (aged 4,  $4\frac{1}{2}$ ,  $5\frac{1}{2}$  (2) and 9 (2) months) has been studied by means of  $100\mu$  horizontal unstained sections, cut by a freezing microtome. Certain lower primates have also been examined.

## RESULTS OF INVESTIGATION

The shaft of the terminal phalanx is not set at right angles to its proximal articular surface, but deviates towards the fibular side of the foot (Fig. 1). In the male students the range of deflexion was 8–23° (mean 14·7°, s.d. 4·1); in the female students 10–22° (mean 14·7°, s.d. 4·3); and in the cadavers 3–25° (mean 14·4°, s.d. 4·5). Though the distal articular surface of the *proximal* phalanx is rarely placed exactly at right angles to the shaft of this bone, the deflexion is relatively small.

Histological sections of foetal great toes also reveal lateral deviation of the terminal diaphysis comparable in degree to that noted in the adult (Fig. 2).

This diaphysial angulation appears to have modified the insertion of the flexor hallucis longus, a suggestion prompted by comparison between the bony roughenings for the distal attachment of this tendon and the corresponding tendon in the thumb; the latter has been described in detail elsewhere (Wilkinson, 1953). The shaft of the terminal phalanx of the thumb is usually set at right angles to the interphalangeal joint, though sometimes it exhibits a slight deflexion towards the ulnar side. The flexor pollicis longus is inserted into a curved bony ridge (Fig. 3*a*). Whilst there is a similar arch-shaped roughening for the insertion of the flexor hallucis longus, its apex is displaced towards the fibular side (Fig. 3*b, c*). Moreover, the arch is very strongly marked on the tibial side of the ventral surface and here constitutes an oblique ridge as portrayed by Grant (1947). As this ridge extends distally, with a lateral inclination, it sometimes reaches the terminal tuberosity of membrane bone (*tuberositas unguicularis*), forming a sort of bony ‘flying buttress’ (Fig. 3*c*).

Dissections of the flexor hallucis longus tendon in the adult show that the fibres



proceed more distally on the fibular side than on the tibial side of the plantar surface of the phalanx. Fig. 4 illustrates a frequent arrangement: a sheaf of fibres extends more distally on the fibular side to become attached to the ventral surface of the tuberositas unguicularis. This asymmetry of insertion is not so obvious in the infant.



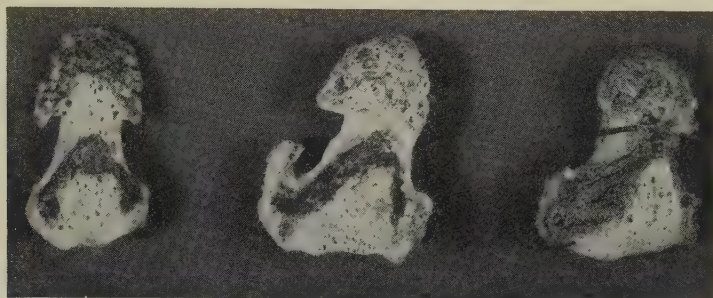
Fig. 1. Radiograph of a right great toe. The shaft of the terminal phalanx is not set at right angles to its articular surface.



Fig. 2. Horizontal section through the right great toe of a 5½-month foetus ( $\times 7.5$ ).

On each side a ligament extends between the base of the bone and the tuberositas unguicularis comparable to that present in mammal digits. It provides a partial, and sometimes extensive, insertion for the tendon. The attachment of this ligament to

the base of the terminal phalanx is not uncommonly indicated by a bony projection (Fig. 3*b*).



(a)

(b)

(c)

Fig. 3. Terminal phalanx of a thumb (*a*), and of each of two left great toes (*b*, *c*). The arch-shaped roughening for flexor tendon insertion has been marked with indian ink. In (*c*) this ridge reaches the tuberositas unguicularis, spanning a small foramen in which a bristle has been placed.



Fig. 4. Dissection of the insertion of flexor hallucis longus in a right great toe. The insertion extends more distally on the fibular side ( $\times 1.5$ ).

As in the thumb, the extensor tendon of the terminal phalanx of the great toe has a more proximal insertion than the corresponding flexor (Fig. 5); this is probably related to the extent of the nail bed. The tendon of the extensor hallucis longus is inserted proximal to the site of diaphysial angulation.

## DISCUSSION

The importance of the human great toe is reflected in the commonest pedal digital formula. The size of the long flexor tendon, greater in cross-section than that of any other human digital flexor, necessitates extensive distal attachments. These are provided by the base and shaft of the terminal phalanx, and often by the tuberositas unguicularis, the ligaments which lie on each side between this and the base of the bone, and by the fascial trabeculae of the fatty pulp. The position of insertion of the



Fig. 5. Sagittal section of the great toe of an adult. (e) insertion of extensor hallucis longus, (f) insertion of flexor hallucis longus (Verhoeff) ( $\times 2$ ).

flexor hallucis longus is commonly portrayed incorrectly in anatomical illustrations. Thus Testut (1928) and Jamieson (1947) confine it to the base of the terminal phalanx.

A comparison with the insertion of the flexor pollicis longus suggests that the insertion of the flexor hallucis longus tendon has been modified by two factors: (1) the necessity for an extensive distal attachment, and (2) the maintenance of an alinement between the tendon and the toe despite a terminal diaphysial deformity.

The deviation of the shaft towards the fibular side of the foot—commonly featured in anatomical atlases—is not primarily caused by the adoption of unsuitable footwear, for it was well established in the foetuses examined; it is not more marked in one sex; it cannot be related to the oblique pull of the long extensor tendon nor is it a feature peculiar to hallux valgus. The reasons for its presence are rather obscure. In the material studied, a fibular-sided deflexion was found only in feet whose structure has been adapted for orthograde locomotion. Thus it was absent in a chimpanzee examined, and there was a deviation towards the tibial side in a baboon and a rhesus monkey. In the 'take-off' position the human foot is usually pointed outwards and pressure is brought to bear on the medial border of its distal extremity. As an inherited structural adaption to function, such a factor may explain this curiously constant bending of the terminal phalanx of the great toe.



SUMMARY

The diaphysis of the terminal phalanx of the great toe deviates towards the fibular side of the foot in all the adult and foetal human material examined, and this deflexion represents the normal condition in man. The feet of other primates studied have not shown this feature. The insertion of the flexor hallucis longus appears to have been modified by this bony conformation. The corresponding extensor tendon is inserted proximal to the site of angulation.

I wish to thank Prof. G. A. G. Mitchell for his interest and helpful criticism, Mr F. L. Newell, the radiographer, and Mr P. Howarth, the photographer, of the University Anatomy Department. The Medical Research Council has defrayed expenses involved in this study.

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## IN MEMORIAM

PROFESSOR J. P. HILL, F.R.S.

Professor J. P. Hill died very suddenly on 24 May 1954. Although in his eighty-first year his mind was alert and active and his critical faculties were unimpaired. He was hard at work to the last hour as he would have wished.

In Volume 82, 1948, of the *Journal of Anatomy*, which was dedicated to J. P. Hill, there was an account of his career and an appreciation of his work including a full bibliography. The list of publications extends over a long period, from 1892 to 1947, and is evidence of a life of continuous study and careful observation of morphological and developmental problems. Since 1947 he published a paper\* in conjunction with G. R. de Beer. Another paper,† with W. C. Osman Hill as collaborator, is in the press. His valuable observations and descriptions of the origin and fate of the neural crest cells, to which he devoted so much time in his last few years, had, unfortunately, not advanced far enough for publication.

Professor Hill's comprehensive knowledge of the animal kingdom, both invertebrate and vertebrate, was outstanding. He was a great teacher and a patient demonstrator, always ready to explain difficulties to his students and he gave them the benefit of his skill in technique and his mastery of diagnosis.

Vertebrate embryology as he taught it was a first-class training; it was a unique education in accurate observation and sound judgement. His microscopic preparations were superb and he had a genius for correctly interpreting the details in his sections, an interpretation which carried conviction. A series of notebooks in which the details of every embryo were meticulously recorded, often with an accompanying photograph, form an invaluable guide to his extensive collection.

Whilst J. P. Hill's own investigations were confined to the morphological aspect, for which he was so supremely qualified, he nevertheless followed the newer methods of experimental embryologists with interest and appreciation. His outlook was a wide one.

It has been my privilege to have worked with Professor Hill for many years; his continual help, valuable advice and wise criticism have been an inspiration to me. His high distinction as an embryologist is universally recognized. Those who knew him best will have memories of his quiet humour and his kindly nature. E.A.F.

\* With G. R. de Beer: 'The development of the Monotremata. VII. The development and structure of the egg-tooth and caruncle in the monotremes and on the occurrence of vestiges of the egg-tooth and caruncle in marsupials.' *Trans. Zool. Soc. Lond.* 1950, 26, 503-544.

† With W. C. Osman Hill: 'The growth-stages of the pouch-young of the Viverrine native cat (*Dasyurus viverrinus*) together with observations on the anatomy of the new-born young.' *Trans. Zool. Soc. Lond.* (in the Press).

## REVIEWS

*Animal Species and their Evolution.* By A. J. CAIN, M.A., D.Phil. (Pp. ix+190; 7½ × 5 in.; 8s. 6d.). London: Hutchinson's University Library. 1954.

The classification and naming of animals has often been regarded as the specialized occupation of museum naturalists, an activity which has little relevance to other branches of biology. It has become increasingly clear, however, as Dr Cain points out, that 'taxonomy is not merely a necessary pigeon-holing but also one of the most important activities in biology, requiring a synthesis of all other biological pursuits for its proper performance, and producing results of the highest importance in the study of evolution'. Moreover, no biologist can afford to neglect taxonomy completely, since the proper naming of animals is an essential pre-requisite to their scientific study and much confusion may be created by errors in identification.

In the first four chapters Dr Cain gives a lucid account of the methods and aims of modern zoological classification which seeks to express the genetic affinities of animals as they have resulted from the historical process of evolution. Questions relating to the rank in the taxonomic hierarchy which should be given to various groups of animals, and to the rules of nomenclature and priority are discussed. His extensive use of mammals, notably Carnivores and Primates, as examples will be appreciated by anatomists.

The remainder of the book is devoted to the concept of the species, a category which is of prime importance from the evolutionary standpoint, and one which must be reconsidered in the light of recent advances in genetics, palaeontology and other fields of knowledge. The term species has come to be used in a number of different senses. There is, for example, the morphological species, a category which may be based solely on the anatomical characters of a museum specimen, and the biological species, definable as a group of 'actually or potentially interbreeding populations which are reproductively isolated from other such groups' (Mayr). The relationships and distinctions between these and other kinds of species are discussed by Dr Cain at some length.

This book provides a valuable introduction to the principles of taxonomy, and a survey of current views on the nature and evolutionary importance of the species.

A. D'A. BELLAIRS

*Man's Ancestry. A Preview of Human Phylogeny.* By W. C. OSMAN HILL, M.D., F.R.S.E. (Pp. ix+194; 100 figs; 21s. net.) London: William Heinemann Medical Books, Ltd. 1954.

This small volume is based on a series of lectures delivered by the author to students at Edinburgh University. It is intended as an introduction to human phylogeny for those studying social anthropology and archaeology or proceeding to more advanced courses in zoology, anatomy and physical anthropology. The scope of the book fulfils this ambitious intention. After a concise presentation of the evidence for evolution there follows an outline of the evolution of the chordates and of the horizons within the chordate phylum. These are dealt with within about 35 pages and consideration of many topics is necessarily short; for example, changes in dentition and in the jaw mechanisms occurring in the transition from reptiles to mammals receive the briefest mention. There follows an elementary account of the nervous system from an evolutionary standpoint as an introduction to the primates. Here existing subhuman primates and the fossil types of significance are dealt with in about 40 pages, leaving a little over one-third of the book for the history of the human stock. To conclude this part man's relation to the apes and the factors involved in the emergence of man are briefly discussed. It is regrettable that the bearing of genetics on anthropology has found no place in this book. Even a brief discourse of this aspect of the problem would have been welcome. However, the reader will find compensation for this deficiency in the excellent account of fossil man, together with the relevant geological and other evidence bearing on this. The treatment here is similar to that found in the excellent little booklet



by Dr W. L. H. Duckworth on 'Prehistoric Man'—a work which unfortunately has not been re-edited and brought up to date.

Professor Osman Hill can be complimented on the production of a really useful introduction to anthropology with an excellent bibliography as a guide for further reading by more advanced students. Though the presentation is simple and clear, it is doubtful whether the claim that, with the aid of the glossary it should be readily understood even by the 'intelligent layman' will be substantiated. This is clearly not the main object of the book, which will undoubtedly be much appreciated by undergraduates embarking on a course of anthropology. It can be warmly commended to them as well as to others interested in the subject.

D. V. DAVIES

*Anatomy and Surgery of Hernia.* By LEO M. ZIMMERMAN, M.D. AND HARRY J. ANSON, PH.D., (MED.SC.) (Pp. x + 374; 204 figs.; 76s. 6d.) London: Baillière, Tindall & Cox. 1953.

This volume is the combined effort of an anatomist, already well known for his investigations into the anatomy of the hernial regions, and a surgeon. This review will be confined mainly to the anatomical aspects of the work as the surgical parts will undoubtedly be dealt with in more appropriate journals.

The book opens with a brief historical account. In view of the widespread incidence of hernia it always seems disappointing that the topic does not appear to figure in the medical literature before the time of Celsus in the first century A.D. The second chapter deals with hernia in general, the aetiology and pathology, especially of strangulation and treatment. The authors stress that hernia must be thought of as 'a hole or a defect', but are not consistent on this in later parts of the book where the 'protrusion' again comes to the fore. In this chapter the incidence of hernia, both relative and absolute, is considered. The geographical distribution of hernia, so well collated by Hirsch, is not considered or mentioned.

Approximately 50 pages are devoted to the anatomy of the abdominal wall. Most of the information here can be found in any sizeable text-book of anatomy. The authors have, however, devoted some space here and elsewhere to the variations which occur, as, for example, the extent of the fleshy parts of the abdominal muscles, the form and position of the linea semicircularis and of the 'inguinal triangle'—the deficiency below the lower margin of the internal oblique muscle. They stress the importance of those congenital, and what is believed may be hereditary, variations in the aetiology of hernia.

In the remainder of the book each type of hernia with its relevant anatomy, pathology and treatment is dealt with individually. The authors believe that both the indirect and direct inguinal types depend on a congenital anatomical disposition. It was Camper—a Dutch physician of the eighteenth century, who pointed out that the processus vaginalis on the right side is nearly always open at birth, whereas that on the left is usually closed. Luther Holden saw in this the explanation of the greater frequency of hernia on the right side in children under 1 year and quoted the figures of the time given by the City of London Truss Society, where in 3014 cases of inguinal hernia under 1 year, 2269 were right sided and 745 were on the left—a proportion of 3 to 1. More consideration could well be given to laterality in this volume. The chapter on diaphragmatic hernia is by Dr R. B. Bettman. Here the anatomy and embryology of the diaphragm cannot be considered in any way exhaustive or even adequate.

The whole book is essentially a reflexion of American teaching. There is little reference to British anatomical teaching. Thus, Lunn's work on inguinal hernia is not mentioned, Lytle's work is omitted and the classical work of Astley Cooper receives but the briefest mention, whilst no reference is made to Keith and Low in the section on the diaphragmatic hernia. The publishers are to be complimented on the excellence of the production. The book is beautifully illustrated, mostly from original dissections. The terminology is mainly but not consistently the Latin form of the B.N.A. in the index, though some terms which we associate essentially with hernia, such as the canal of Nuck and Hesselbach's triangle, cannot be found in the index; they appear however in several places in the text.

D. V. DAVIES

## PROCEEDINGS OF THE ANATOMICAL SOCIETY

NOVEMBER 1953

The Annual General Meeting of the Society for the Session 1953-4 was held on Friday, 27 November 1953, in the Department of Anatomy, St Bartholomew's Hospital Medical College, Charterhouse Square, London. During the meeting the Past President (Prof. W. E. LE GROS CLARK) vacated the Chair in favour of the newly elected President (Prof. W. J. HAMILTON).

The following are the authors' abstracts of the papers read.

**Postnatal development of the costal cartilages.** By A. J. E. CAVE and N. A. GREEN.  
*St Bartholomew's Hospital Medical College, London*

Up to 30 years of age the costal cartilages remain unossified as homogeneous, more or less translucent, structures. But at the beginning of the fourth decade their ossification begins in a manner less haphazard perhaps than is customarily described. Each cartilage undergoes a permanently incomplete ossification in two planes—deep and superficial. Deeply (i.e. in the interior of the cartilage) calcification proceeds, taking the form of a beaded or granular central 'core', which, despite gross increase with advancing age, retains its general appearance and independence. Superficially, true bone is deposited in two distinct situations—(a) as an osseous 'cap' on the juxta-sternal extremity of the cartilage, (b) in the form of irregular, variable plaques on its upper and lower borders. The juxta-sternal 'caps' appear first anteriorly, then posteriorly: they remain discrete until the eighth decade when they tend to become confluent with the progressively spreading plaques. These last appear generally about the 50th year (sometimes earlier), and extend in a sustained but unsuccessful attempt to enclose the entire surface of the cartilage. They frequently achieve continuity with the juxta-sternal 'caps', but remain independent of the central 'core'. No costal cartilage ever becomes truly and completely ossified. The relation of this ossification pattern to functional requirements is discussed.

**Parturition in the rabbit.** By K. J. FRANKLIN and N. E. WINSTONE. *St Bartholomew's Hospital Medical College, London*

At term the doe is nesting and restless. She gives birth in the prone position with the body raised off the ground (forelimb extension specially marked) and arched, and the head turned downwards and backwards between the forelimbs; if necessary, one forelimb is raised to allow the head to be still nearer the external os. In this posture the direction of the birth canal is such that the foetus during birth is propelled downwards and forwards between the somewhat splayed hindlimbs, i.e. comes to rest near the doe's mouth so that she can attend to it as required. Placentophagia is usual.

Uterine activity (which is accompanied by increased intestinal activity and reflex and hormonal reduction in the renal cortical blood volume) can on occasion *per se* ensure parturition, but normally skeletal muscles also are reflexly involved. With a slight time-lag, the uterine contractions and relaxations are paralleled by rises and falls in arterial blood pressure. In the anaesthetized doe uterine activity alone can cause rises of the order of 15 mm. Hg, with skeletal muscle synergy of the order of 25 mm. The pressure as a whole rises up to the first birth, falls a little during subsequent births, and remains above its initial value while the uterus is contracting down. The cornua contract down more rapidly than the common portion, and during the process there is continuance of effect upon the renal cortical vasculature.

**The development of the dural venous sinuses in man.** By H. BUTLER.  
*St Bartholomew's Hospital Medical College, London*

The generally accepted account of the development of the human dural venous sinuses is that of Streeter (1915). According to his scheme the middle cerebral vein, which joins the primary head vein between the trigeminal ganglion and the otocyst, becomes the superior petrosal sinus. Also, this scheme takes no account of the petro-squamous sinus, although Markowski (1911) had shown that this sinus is developed from the middle cerebral vein. A personal investigation of the development of the dural venous sinuses of the rat showed that the middle cerebral vein became the petro-squamous sinus, not the superior petrosal sinus. Since the middle cerebral vein has an identical position in both the rat and the human embryo, its fate in the latter has been re-examined.

It was found essential to base the homologies of the various embryonic and adult head veins upon their relationship to cranial nerves, other than and additional to the trigeminal. The middle cerebral vein passes between the trigeminal ganglion and the greater superficial petrosal nerve to join the vena capitis medialis (the future cavernous sinus). The adult superior petrosal sinus passes between the trigeminal ganglion and cranial nerves III and IV to join the cavernous sinus. By means of these nerve-relationships it can be shown that, in man, the middle cerebral vein becomes the petro-squamous sinus and that, in embryos of 40–45 mm. C.R. length, it is almost as large as the sigmoid sinus. The petro-squamous sinus receives the middle meningeal veins and acquires a connexion with the pterygoid venous plexus by an emissary vein running alongside the middle meningeal artery. That part of the petro-squamous sinus between the transverse sinus and the posterior group of middle meningeal veins gradually disappears and the remainder becomes the common stem of the middle meningeal veins, communicating with the cavernous sinus and with the pterygoid venous plexus. The superior petrosal sinus is formed from a leash of small veins which runs forwards and ventrally from the middle cerebral vein, passing between the trigeminal ganglion and cranial nerves III and IV, to join the vena capitis medialis. This leash does not form a distinct single channel until the embryo is nearly 100 mm. C.R. length.

**The blood supply of the facial nerve.** By MICHAEL J. BLUNT. *Royal Free Hospital School of Medicine, London*

The blood supply of the facial nerve has been investigated, using human adult and foetal temporal bones. Some of the material was injected and in other specimens the nerve has been dissected out and stained by the Pickworth method. Serial sections of foetal temporal bones have been examined.

The arterial supply to the facial nerve comes from the following sources:

- (1) *Intra-cranial Part*, from the anterior inferior cerebellar artery.
- (2) *In the Internal Auditory Meatus*, from the branches of the internal auditory artery.
- (3) *At the Geniculate Ganglion*, from the petrosal branch of the middle meningeal artery.
- (4) *Between the Ganglion and the Stylomastoid Foramen*, from the petrosal artery, the stylomastoid artery and the arterial arcade formed by the anastomosis of these two vessels.
- (5) *Between the Stylomastoid Foramen and the Bifurcation*, by branches from the occipital or posterior auricular arteries.
- (6) *In the Parotid Gland*, by branches from the occipital or posterior auricular arteries and from the superficial temporal and transverse facial arteries.

This communication is particularly concerned with the vascular arrangements between the geniculate ganglion and the bifurcation of the facial nerve. The gross and microscopic form of the blood supply are described in detail.



**The size of the ductus venosus.** By A. D. DICKSON. *The Queen's University, Belfast*

The diameters of the umbilical vein near its termination, and of the ductus venosus at its origin and termination, were measured in a series of human embryos and fetuses. Most measurements were made on serial sections, but some were made on neoprene casts of the hepatic vessels. The results showed that the termination of the ductus venosus was larger than the origin, and that the origin was, at all ages, considerably smaller than the umbilical vein (the average ratio of the cross-sectional areas was 1:7). The significance of the results, in relation to the problem of the proportion of the umbilical blood which traverses the ductus venosus, was discussed.

**Variations in the blood supply of the jejunum.**

By T. E. BARLOW. *University of Durham*

Recent operations on the jejunum (Robertson, R. & Sargeant, T. R., 1950) have involved the division of the main jejunal branches of the superior mesenteric artery prior to using a length of jejunum to replace an oesophagus rendered useless by constricting growths. The jejunum so used is entirely dependent upon the arcade system for its blood supply. Some variations of the arcade formation have been noted which are important in view of the fact that this operation is becoming more widely adopted. Observations made show that the arcade system is by no means always continuous, that there may be a complete gap between two of the upper jejunal arteries in the mesentery, the only anastomoses between them being within the wall of the jejunum. Occasionally the lateral anastomoses between the upper jejunal branches in the mesentery are very slender and not at all like the usual large vessels portrayed as being the normal arrangement. In the specimens examined so far, twelve of which were X-rayed, the vessels being previously injected with 20 % chlorbismol, one showed a complete gap in the arcade system and another had a very slender twig between the first and second jejunal arteries. The consequences of a break in the arcade, should operation become necessary, are obvious and it is important to find out in what percentage of subjects it occurs. Probably the quickest way of obtaining figures is to ask Members of the Society to make observations in their own schools upon dissecting room cadavers. This could easily be done by the students as the mesentery is dissected and the figures so obtained would form a valuable addition to the surgeon's knowledge of the variations he is likely to meet.

**The effect of phosphorylated hesperidin on the growth rate and fertility of pre-pubertal rats.** By P. BACSICH and A. YOUNG. *University of Glasgow*

It was reported (Martin, G. J. & Beiler, J. M., *Science*, **115**, 402, 1952) that oral, or parenteral administration of phosphorylated hesperidin to male and female rats prevents fertilization through the inhibiting action of this new drug on the enzyme hyaluronidase. These results were also substantiated by an extensive clinical trial on human material (Sieve, B. J., *Science*, **116**, 373, 1952).

In the present experiment phosphorylated hesperidin (provided by the National Drug Co., U.S.A.), was administered orally to a group of male and female prepubertal rats. All animals, including controls, were litter-mates. Owing to the probable usefulness of this drug in the treatment of rheumatoid arthritis (Bacsich, P., *Brit. Med. J.* **2**, 231, 1952), and in order to ascertain that it could be safely administered to growing individuals, our interest centred principally on the possible effect, if any, of the above chemical on the enzymatic mechanism of the epiphyseal cartilage, and thus on the growth rate of the animals. Observations were also made on the fertility rate, as all animals have reached sexual maturity during the experiment.

No significant difference was observed either in the growth rate, or in the fertility of the animals; on the other hand there was a statistically significant neonatal mortality in the progeny of the treated group. Some theoretical and practical aspects of these findings were discussed and correlated with statements in the recent literature.

**Some recent observations on the effects of hypophysectomy on skeletal morphogenesis in rats.** By C. WILLET ASLING. *University of California, Berkeley, U.S.A.* (introduced by R. G. HARRISON)

Detailed analysis of the consequences of hypophysectomy on rats' skeletal development demonstrates the inadequacy of statements referring simply to 'arrested growth'. Data from recent experiments support the following views:

- (1) The extent of retardation of growth is dependent upon the age at hypophysectomy. Impairment is less in younger than in older rats, suggesting that growth of the very young animal is relatively less dependent on pituitary hormones.
- (2) The extent of retardation of maturation (judged by epiphyseal differentiation) is independent of the age at hypophysectomy. The equivalent of 2-3 weeks of further differentiation follows hypophysectomy of immature rats of whatever age.
- (3) The proportions existing between lengths of the various bones are altered, so that long after hypophysectomy of growing rats these proportions are found to be neither those existing at the time of operation nor those characterizing adults. Differences in the growth curves of individual bones are a major factor in accounting for these ultimate disproportions.
- (4) There is some evidence that neither growth nor maturation of the skeleton is absolutely arrested following hypophysectomy, but instead are reduced to a rate so low that only prolonged observation can detect the slight advances.
- (5) A remarkable mortal consequence of hypophysectomy shortly after birth can be demonstrated and related to imbalance in effects on skeletal and neural growth.

**The relationship of odontoblasts to tooth dentine.** By W. WARWICK JAMES.  
*Middlesex Hospital Medical School, London*

Dentine is an acellular tissue consisting of a calcified matrix permeated by tubules which are said to contain dentinal fibrils from the odontoblasts. The matrix contains collagen fibrils which constitute 18 % of its weight, 75 % being inorganic (Stack, 1951).

No satisfactory illustrations showing the connexion between dentinal fibrils and odontoblasts can be found in the literature; moreover, the appearance of fibrils within a dentinal tubule in section may be due to the presence of coagulated colloidal material, or even to an optical effect caused by focusing, rather than the presence of a true fibril.

Re-examination of both mammalian and non-mammalian dentine does not confirm the presence of processes from the odontoblasts within the tubules. Carefully prepared sections of developing teeth from animals ranging from fishes to mammals show odontoblasts connected with the matrix of the predentine, the tubules being continuous with the spaces between the odontoblasts. In non-mammals odontoblasts may be fusiform with fibres parallel to their long axes passing into the matrix; in mammals the distal end of an odontoblast is square and under high magnification appears to be open, with the granules in the cytoplasm continuous with those in the developing predentine.

Collagen fibrils can be seen in the matrix of developing dentine, especially round the periphery of a tubule; these fibrils may project from the dentine when the cells are pulled away, and have been interpreted as broken-off dentinal fibrils, e.g. by Tomes (1856). Von Ebner (1891), Mummery (1892) and von Korff (1905) have recognized that collagen fibrils of the pulp are continuous with those in the dentinal matrix, and that they pass along the odontoblasts.

It is concluded that odontoblasts have a similar function to fibroblasts in the production of collagen fibrils and that the tubules correspond with tissue spaces and contain fluid.

**The effects of some mitotic inhibitors on the early development of chick embryos.**  
By RUTH BELLAIRS. *University College, London* (introduced by M. ABERCROMBIE)

The effects of administering certain substances which inhibit mitosis have been studied in early chick embryos. The inhibitors used were aminopterin, colchicine and 'Synkavit',

an analogue of vitamin K. They were introduced into the egg through a hole in the shell. The influence of these substances on the differentiation of the neural tube, heart and foregut and on the extension of the blastoderm over the surface of the yolk is discussed. It is suggested that local variations in the rate of mitosis play an important part in differentiation, but not in the extension of the blastoderm.

### **Electron-microscopic studies of spinal ganglia cells in the rat.**

By G. M. WYBURN. *University of Glasgow*

Osmic acid fixed sections were cut 0.2 to 0.1  $\mu$  thick and observed and photographed with the electron microscope. The special features of interest are the nuclei, the nucleolus, the nuclear membrane and the structure on the cytoplasm. There is some indication of a difference in the cytoplasmic structure in the various cells. Other points of interest are the structure of the nerve fibres and the nucleated capsule.

### **The effect of fixation on neurones of the chick.**

By ARTHUR HUGHES. *University of Cambridge*

The action of different fixatives has been studied in some developing neurones, which have also been observed unfixed under the phase microscope. Purkinje cells of the chick at hatching have been examined in squashes of the cerebellar folia. Unfixed, the cytoplasm of the perikaryon is seen to be filled with granular or rod-like inclusions. The same appearance is seen in sections of the cerebellum fixed by the freeze-drying method, or by osmic acid. Silver impregnation after freeze-drying shows that much of this granular material is argyrophilic. Fixation in Carnoy's fluid, followed by silvering, produces the conventional picture of a neurofibrillar network. The conclusion has been reached that this network is formed at fixation by vacuolation in the cytoplasm. The production of these vacuoles has been followed in unstained fragments of the cerebellum squashed in various fixatives. The size of the vacuoles closely follows that of the neurofibrillar meshes when the same fixative has been used. Similar results have been obtained with chick spinal ganglia, in which it is known that most of the cytoplasmic inclusions within the perikaryon are mitochondria. Recently, this study has been continued with the aid of the electron microscope, and preliminary results obtained are demonstrated.

### **The identity and function of the posterior dorso-central nucleus of Panegrossi.**

By R. WARWICK. *University of Manchester*

Panegrossi (1898, 1904) and Tsuchida (1906) have described independently a median nucleus of motor cells of intermediate size, situated dorsally in the caudal third of the oculomotor complex of both man and monkey. They named it respectively 'nucleus posterior dorso-centralis' and 'caudal central nucleus'. Panegrossi's term has also been applied by some authorities to a median nucleus rostral to Perlia's. Inspection of serial sections of the oculomotor complex in one human, two chimpanzee and fifty-three monkey mid-brains revealed no evidence of this rostral group. In the caudal third of the complex no other median group, except the dorsal nucleus of the raphe, was observed; and from their accounts this could not correspond with the nucleus referred to by Panegrossi or Tsuchida. Moreover, the descriptions of both workers were practically identical, and tallied in all details with the observations of this research upon the caudal central nucleus. In view of this, and of the confusion which has arisen in the use of Panegrossi's term, it is preferable to adopt Tsuchida's designation, the 'central caudal nucleus'. Experimental data, derived from observations on the retrograde changes produced by selective oculomotor lesions, have shown that the central caudal nucleus innervates both levator muscles, a finding in agreement with the bilateral nature of the ptosis caused by nuclear disease.



**The origin of the mamillo-thalamic tract in the rat.** By T. P. S. POWELL  
and W. M. COWAN. *University of Oxford*

In an experimental study of the fornix system of the rat the anterior thalamic nuclei were in a number of cases incidentally damaged. By correlating the extent of this damage with the resulting retrograde cell degeneration in the mamillary nuclei information concerning the origin of the mamillo-thalamic tract has been obtained. The tract arises from all elements of the medial mamillary nucleus (except the pars medianus) and possibly from the premamillary nuclei; no contribution is made by the lateral mamillary nucleus. There is a precise projection from each element of the medial mamillary nucleus to each of the anterior thalamic nuclei: the pars medialis projects to the antero-medial nucleus, the pars lateralis to the antero-dorsal nucleus and the pars posterior to the antero-ventral nucleus.

**The elastic nature of the articular mechanism at the human knee joint.**  
By J. W. SMITH. *University of St Andrews*

The tissues involved in any particular mechanism acting at a synovial joint are elastic in the physical meaning of that term; they are distorted by stress and within certain limits recover their original form when the stress is removed. It is suggested that because of the elasticity of the articular tissues an articular mechanism will not necessarily arrest movement at a joint at one particular position but will operate in an elastic manner. Thus it is suggested that when a very small force acts on the joint the articular mechanism will arrest movement at a position short of full extension, but if a greater force acts the restraining joint tissues will be distorted and further movement at the joint will be allowed. Furthermore, this movement at the joint will be elastically resisted by the distorted joint tissues and will therefore persist only as long as the force producing it persists.

The existence of such an elastically resisted range of movement has been confirmed and examined in the terminal phase of passive extension of the living human knee joint; it extends approximately over the terminal 15° of voluntary extension.

An attempt has been made to correlate these findings with certain functional aspects of the movement at the knee joint.

**The morphological significance of fusion between manubrium and corpus sterni.** By G. T. ASHLEY. *University of Manchester*

The following conclusions are arrived at from a review of the literature and personal study of 1400 sterna ranging in age from four months (foetal) to advanced senility.

(1) The accepted teaching that synostosis of the manubrio-sternal joint is rare and that it is a senile change should be revised.

(2) Conclusive evidence is presented to show that such synostosis is found in no less than 10% of adults and that it is equally common in all 10-year age groups after the age of 30.

(3) The synostosis would seem to be of two kinds:

(a) Primary, 'matrical' synostosis, resulting from the obliteration, during early life, of a *primary* cartilaginous joint between manubrium and corpus sterni and presumably more or less inevitable.

(b) Secondary, 'sclerotic' synostosis, resulting from the obliteration during late adult life, of a *secondary* cartilaginous joint between manubrium and corpus sterni, and presumably the result of pathological processes.

(4) In 683 adult sterna of known age, the primary of 'matrical' type of synostosis was found three times as commonly as the 'sclerotic' type.

**Direct observation of changes in tension in the supraspinous and interspinous ligaments during flexion and extension of the vertebral column in man.**

By P. H. S. SILVER. *Middlesex Hospital Medical School, London*

When the vertebral column is fully flexed, e.g. in toe touching, the erectores spinae relax. The presumption has been made that, in the fully flexed position, trunk posture is

maintained largely by tension in the intervertebral ligaments. This paper provides direct evidence to support this presumption.

Under local anaesthesia a pin was driven horizontally into the supraspinous and interspinous ligaments between the spines of the 3rd and 4th lumbar vertebrae. The pin was then pulled laterally first to one side and then to the other by means of a spring balance. The amplitude of the movement in the coronal plane was measured by means of a graduated slider in which the base of the pin was held. Erectores spinae activity was recorded simultaneously by electromyography.

In the extended position the ligaments were slack, and the pin could be moved freely. As trunk flexion took place the amplitude of sideways movement of the needle diminished, and eventually disappeared as the ligaments came under tension due to the separation of the lumbar spines. The development of tension in these ligaments never coincided with the flexion-relaxation of the erectores spinae, but always preceded it. Then, with further trunk flexion, the tension in these ligaments increased, and the activity in the erectores spinae diminished and finally ceased. The period of overlap between the development of tension in these ligaments and the cessation of erectores spinae activity is accounted for by the presence of the elastic fibres, which have been demonstrated histologically, both in and around the supraspinous and interspinous ligaments.

### **The effect of temporomandibular meniscectomy in rabbits.**

By R. SPRINZ. *Royal College of Surgeons*

Regeneration of the menisci of the knee joint in rabbits has been demonstrated by Walmsley and Bruce (*J. Anat., Lond.*, 72, 1938). The suggestion that similar regeneration occurred in the temporomandibular joint has not been supported by the evidence from human material. In view of the importance of this joint on balanced dental occlusion, the effects following excision were studied.

Rabbits of varying ages, from the newborn to the adult, were operated on. The post-operative condition was studied with reference to the well-being of the animal, and the effect of the operation on the dentition.

No regeneration of the meniscus of the temporomandibular joint in rabbits could be demonstrated. The place of the meniscus was taken by an enlargement of the condylar head of the mandible.

As the menisci regenerate in the knee and not in the temporomandibular joint, a difference in the function of the menisci is suggested.

### **The mechanism of the ear-flaps in Crocodilia.** By C. C. D. SHUTE (*University of Cambridge*) and A. D'A. BELLAIRS (*St Mary's Hospital Medical School, London*)

The outer ear of crocodiles and alligators is found to be guarded by an upper flap which is normally kept closed, but which can be opened, as when water has entered the meatus; and by a lower flap which is usually open to permit the entry of sound waves, but which can be closed, as when the animal dives. Both flaps are supported by condensations of connective tissue which give attachment to special muscles. The muscles associated with the upper flap are situated on its deep aspect; those acting on the lower flap are related to the orbit. The detailed attachments of these muscles are described, and a theory offered as to how they are able to bring about the movements of opening and closure.

### **Some observations on the bronchial system of the common seal.**

By D. BROWN. *Charing Cross Hospital Medical School, London*

Bronchial corrosion casts of two specimens of the lungs of the common seal (*Phoca hispida*) were prepared as part of a mammalian series by a modification of the Tompsett technique, using Marco resin S.B. 26C.

The casts demonstrate several unusual features. Whereas the lungs of all the terrestrial mammals, excepting man, seem to show an unpaired right cardiac lobe between the heart and the diaphragm, in the common seal this is not present; furthermore the bronchial casts demonstrate a complete bilateral symmetry, again an unusual feature in mammalian lungs.

The bronchial system is compared with that of the typical fissipedia and some observations are made on the significance of symmetry in the lungs of aquatic mammals.

**The structure of the pinniped lung.** By N. C. D. PIZEY.

*London Hospital Medical College*

A brief description of some of the macroscopic and microscopic features of *Phoca vitulina* is given.

The whole lung is seen to be divided into lobules by well-marked septa, and the presence of elastic tissue is a feature. A note is made of the rich subpleural vascular plexus.

The absence of glandular tissue in the trachea in contrast with abundant glands which are found in relation to the smaller bronchi and bronchioles, and which are outside the cartilage, is commented on.

Between the epithelium and the cartilage of the trachea there are large dilated lymph spaces. In the smaller divisions of the bronchi a subepithelial vascular network is present.

A series of valves in the bronchioles, noted in some other diving mammals, is shown.

In addition to these histological features attention is drawn to numerous patches of inflammation and the presence of parasitic worms in many of the sections.

The functional significance of some of these findings is discussed.

**The histochemistry of the normal mouse prostate and changes following castration, application of oestrogenic substances, application of homografts, and in experimental carcinogenesis.** By D. BRANDES and G. H. BOURNE. *London Hospital Medical College*

The periodic acid-Schiff reaction in the mouse prostate was localized in the basement membranes, in the secretion, and in the small granules in the cytoplasm and in the Golgi region. Acid phosphatase was localized in the nuclei and in the Golgi region. Alkaline phosphatase was in the basement membrane and in the endothelium of the small blood vessels. The lipase reaction was negative. The Golgi region stained black with Sudan Black but there was no material in the cells stainable with Sudan IV.

Twenty-two days after castration, PAS-positive material almost disappeared. Acid phosphatase was still present in the nuclei but in the anterior lobe was not present in the Golgi region. The Golgi apparatus was fragmented in this lobe, partly fragmented in the ventral lobe and normal in the posterior lobe. The alkaline phosphatase reaction decreased. Testosterone implants into castrates returned the Golgi material and the histochemical reactions to normal. Implantation of oestrogenic substances caused first an increase, then a decrease in PAS-positive material with an accumulation in the Golgi region and a loss of acid phosphatase from the Golgi region in the anterior lobe; the Golgi material was fragmented in this lobe. Acid phosphatase later almost disappeared from the nuclei and the alkaline phosphatase reaction decreased.

Eight days after removal of the oestrogenic material the reactions were only partly back to normal.

In the anterior lobe following homografting, the PAS-positive material disappeared, the acid phosphatase reaction in the nucleus became feebler and the reaction disappeared from the Golgi region, and the alkaline phosphatase reaction was greatly enfeebled. These reactions returned as the graft took and new secretory acini were formed. With ventral lobe grafts the restitution of the PAS-positive granules as well as acid and alkaline phosphatase was never really complete.



In grafts impregnated with methyl-cholanthrene, squamous cell metaplasia developed. During this process the PAS-positive material disappeared from the cells, the acid phosphatase reaction decreased and the alkaline phosphatase reaction disappeared. Some grafts underwent malignant transformation with the production of squamous cell carcinomas. There was almost complete loss of the normal histochemical reactions in these cases.

The conclusions are that the Golgi material and the PAS-positive material and acid and alkaline phosphatases are susceptible to hormonal control and that they undergo profound modification in prostatic carcinogenesis.

#### Some observations on the development of the pharyngeal derivatives in birds.

By R. J. SCOTHORNE. *University of Glasgow*

The development of the pharyngeal derivatives has been studied in a series of embryos of the Black-headed Gull (*Larus ridibundus*) and of the Gannet (*Sula bassana*).

In both species a diverticulum develops from the caudal part of the primitive pharynx, in common with the IVth pouch. This caudal diverticulum subsequently becomes partially subdivided, the lateral moiety apparently representing a Vth pouch, the medial moiety, which occupies the position of a VIth pouch, forming the principal anlage of the ultimobranchial body. The subdivision of the caudal diverticulum is more conspicuous in the Gull, but the so-called Vth pouch does establish transient ectodermal contact in the Gannet.

The later development of the ultimobranchial bodies is asymmetrical in both species, that on the left being much larger than that on the right.

Parathyroids develop from pouches III and IV. The thymus is almost entirely a IIIrd pouch derivative, thymus IV being rudimentary. In both species there is evidence of abortive thymus development from the IInd pouch.

#### Observations on the origin and fate of the urethral plate in man.

By T. W. GLENISTER. *Charing Cross Hospital Medical School, London*

The caudal parts of thirty-five human embryos (10–50 mm. C.-R.L.) have been serially sectioned and studied to ascertain whether the mode of formation of the penile urethra in man is similar to that described by Barnstein & Mossman (1938) in the squirrel. The urethral plate consists of an epithelial lamella derived from the fused walls of the anterior portion of the pars phallica of the urogenital sinus. This lamella extends beyond the coronary sulcus, to reach the tip of the phallus in embryos of both sexes. The lower margin of the plate is in contact with the epithelium covering the under surface of the phallus and at the anterior end of the plate, the surface epithelium reacts to this contact by proliferating to produce the epithelial tag. Further back, the surface epithelium has been affected by the urethral plate for a longer period and becomes destratified and eventually disintegrates. The portion of the urethral plate in relation to retrogressing surface epithelium becomes bulbous and then disintegrates to form a urethral groove lined with epithelium ultimately derived from the urogenital sinus. The urethral folds which have been developing on either side of the urethral plate and the pars phallica deepen the urethral groove and the junction between sinus epithelium and surface epithelium lies in the wall of the groove and not at the free margin of the folds. In male embryos there then follows closure of the open pars phallica and the urethral groove by a growing together and fusion of the walls of the urethral groove in such a way that only sinus epithelium lines the resulting spongy urethra. Distal to the coronary sulcus the same basic processes are involved but the formation and fusion of the urethral folds in the distal part of the glans proceed at a faster rate than the proliferation and disintegration of the epithelia. In this way surface epithelium comes to be included in the lining of the fossa terminalis. The bulbo-urethral glands develop as outgrowths from the pars phallica near its junction with the pars pelvina.

## FEBRUARY 1954

An ordinary meeting of the Society for the Session 1953-4 was held on Friday, 26 February 1954, at the Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2. The President (Prof. W. J. HAMILTON) was in the Chair.

The following are the authors' abstracts of the papers read.

**The electromyography of the sternomastoid muscle.**

G. CAUSEY and D. SLOME. *Royal College of Surgeons*

The sternomastoid muscle can act upon the occipitoatlantal, the atlanto-axial and the succeeding joints between the cervical vertebrae. With a four-channel Grass pen recorder, electromyographic tracings have been made in a number of different movements. It has been shown that the sternomastoids are active in a wide range of movements of the head and neck, including extension at the occipito-atlantal joint. The relative activity of different parts has been described.

**The submicroscopic structure of degenerating and regenerating nerves.**

By G. CAUSEY and H. J. HOFFMAN. *Royal College of Surgeons*

Some observations on the structure of degenerating and regenerating nerves as shown by the electron microscope were presented. A Metropolitan Vickers E.M.4 microscope was used with a resolving power of 100 Å. Thin sections of 0.1 to 0.05  $\mu$  were used throughout, cut on a modified Spencer microtome with glass knives.

The reproducibility of cytoplasmic and myelin structure in different fixatives was studied in normal nerves. The appearances after fixation in buffered osmic acid, neutral formalin and Zenker's, Orth's and Carnoy's fluids were studied. The appearance of the axoplasm and myelin after such fixation were discussed. Impregnation with phosphotungstic acid was also used.

In degenerating nerves the early disorganization of the fine fibrillary structure of the axoplasm was shown together with the early proliferation of Schwann cells. In regenerating nerves the appearances of small fibres, both myelinated and non-myelinated, was shown, with some comments on the reappearance of organized axoplasmic structure.

Finally some points in the structure of the motor end plate were shown and discussed.

**The membranes at the surface of Golgi bodies in neurons.**

By J. Z. YOUNG. *University College, London*

In cephalopod neurons there are spheres around the nucleus, visible in the unstained teased cell and stainable with neutral red. In hypertonic solutions these bodies first become shrunken and the red matter then takes on the appearance of a crescent or forms granules attached to the side of a faintly stained sphere. These changes are reversible.

The appearances in hypertonic solution suggest that the neutral red stains chiefly the surface layer and that as the droplet shrinks the tangential cohesive forces are not sufficient to maintain the continuity of this outer layer. It first crumples and then loses its membrane-like properties; its molecules slide over each other and it rolls up into granules. The main droplet maintains its integrity, however, and since the phenomenon is reversible there must be a semi-permeable barrier at the inner surface that remains after the outer has shrunk away.

These bodies are therefore covered by double membranes with semi-permeable properties. It has been shown by various workers that mitochondria have double surface layers, and suspensions of isolated mitochondria show capacity for osmotic regulation and operate a sodium pump mechanism. The bodies described in cephalopod nerve cells do not otherwise resemble mitochondria.

Classical methods for staining the Golgi apparatus colour these bodies, revealing either spheres with smooth or crenated walls, or crescents of various types, with the outer and inner components that have been revealed by many workers. These appearances result from distortions similar to those produced by hypertonic solutions. The outer more osmiophil or argentophil component behaves similarly to the layer that stains more deeply with neutral red.

The objects that stain with the Golgi body techniques in cephalopod neurons therefore include spheres, visible in the living state. They have a double surface layer with semi-permeable properties. The material in this part of the cells is complex, however, and requires further investigation, especially by electron microscopy.

**A quantitative study of the cell/fibre relationships in the mamillary bodies of the rabbit and the cat.** By R. W. GUILLERY. *University College, London*

There are slightly less than 100,000 cells in the mamillary body of the cat (77,500 and 87,000) and slightly more than 100,000 cells in the mamillary body of the rabbit (107,000 and 132,000).

In the cat the number of fibres in the principal mamillary tract and the number of fibres in the premamillary fornix are approximately equal to the number of mamillary cells. In the rabbit a similar correspondence holds between the number of fornix fibres and the number of mamillary cells, but only about 75 % of the mamillary cells send their axons into the principal mamillary tract. The mamillary bodies only receive  $\frac{1}{3}$ , or less, of their total afferent fibres through the mamillary peduncle.

The number of fibres in the principal mamillary tract is approximately equal to the number of fibres in the mamillothalamic tract in both the rabbit and the cat. Since the principal mamillary tract also sends a number of fibres into the tegmentum there must be a considerable amount of branching of the individual fibres of this tract.

The number of fibres in the postcommissural fornix decreases markedly between the anterior commissure and the mid-tuberal region (by about 60,000 fibres in the cat and by about 100,000 fibres in the rabbit). These fibres probably end in some part of the hypothalamus and some fibres have been seen leaving the fornix in this region.

**The peculiarities of ciliary ganglion neurons.**

By R. WARWICK. *University of Manchester*

Certain peculiarities of the neurons of the ciliary ganglion have long been known, especially in birds. Their cytons and axons are large, and the latter, if not (as once believed) unique amongst post-ganglionic fibres in being medullated, possess myelin sheaths perhaps more typical of somatic fibres in their thickness. These characteristics have been noted occasionally in mammalian material, but they are rarely referred to in works on human neurology.

Examination of the ciliary ganglion in a series of reptiles, birds, and mammals (including monkeys, apes and man) has confirmed the large size of its cells. In particular, comparison of the cells in the ciliary, sphenopalatine, superior cervical sympathetic and coeliac ganglia of rhesus monkeys has shown that the ciliary ganglion cells are not only uniformly larger than those commonly found in these other autonomic ganglia, but that they also differ in other characters. In the central position of their nuclei and the distribution of their chromatin content they differ from typical autonomic nerve cells and resemble cerebrosplinal motor neurons.

These peculiarities may be a reflexion of functional differences; and in this connexion the striated nature of the intrinsic ocular musculature of reptiles and birds may be recalled, and also the increasing voluntary control of the eyes in mammals. The solution of such issues, however, requires further research.



**Observations on the reaction of the mouse brain to trauma and a note on the influence of cortisone.** By E. J. FIELD. *University of Bristol*

Oligodendroglia has been described as a source of compound granular corpuscle formation following cerebral trauma in the cat. Like the cat, the mouse presents readily stainable oligodendroglia but poorly demonstrable microglia. The brains of fifty-four unmedicated mice, forty-six treated with cortisone and thirty-four controls treated with cortisone suspending medium have been examined at intervals after a puncture wound. Hortega and Giemsa staining has been used as well as the Herxheimer, Globus and Gross methods for fat. It has been found:

(1) There is a striking absence of reaction up to 24 hr apart from some acute swelling of the oligodendroglia in the immediate neighbourhood of the injury.

(2) Thereafter compound granular corpuscles begin to appear but contain no sudanophil material. They are similar to the cells of Hortega's 'fountains' in the neonatal animal and may be called pseudo compound granular corpuscles. It is possible that in both cases they are handling some intermediary product of myelin metabolism.

(3) Oligodendroglia probably makes a small contribution to pseudo compound granular corpuscle formation. The difficulty in assessing this is discussed.

(4) Microglia emerges from the background and is the main source of compound granular corpuscles.

(5) Cortisone induces a delay in pseudo compound granular corpuscle formation and in the appearance of sudanophil material. Since these cells are derived from the local cell population the delay cannot be attributed to diminished vascular permeability. Possible explanations include: (a) direct depression of macrophage activity, (b) diminished permeability of the ground substance of the brain to that material(s), derived from nervous tissue debris, which stimulates microglial mobilization. These possibilities are discussed.

**The rate of renewal of intestinal epithelium.**

By R. M. H. McMINN. *University of Sheffield*

Mitotic counts of epithelial nuclei in the ileum of the cat indicate that 0.95 % of the total number of nuclei are in mitosis at any one time. Similar counts carried out after the administration of colchicine, whose predominant effect is to arrest mitosis in metaphase, have shown that 3.77 % of nuclei are in arrested mitosis 5 hr. after the administration of this drug (McMinn & Mitchell, *J. Anat.* 88, 1954, in the press). From these figures it may be calculated that the duration of a mitotic cycle is approximately 75 min. The distance from the bases of the crypts to the tips of the villi in this region of the small intestine is approximately 900  $\mu$ . Assuming that there is constant mitotic activity in the crypts with cells passing upwards along the sides of the villi from the tips of which they are eventually shed (Leblond & Stevens, *Anat. Rec.* 100, 1948), further calculation indicates that a cell takes 133 hr. to travel this distance. This must also be the time taken for the complete renewal of the epithelial lining of this region of the intestine.

Similar calculations are being carried out for the duodenum of the cat and for the small intestine of the dog.

**Repair in the colonic mucosa of the mouse.** By R. J. O'CONNOR.

*Westminster School of Medicine, London*

In the colon of the mouse necrosis of small areas of mucosa was produced by the application of silver nitrate. If the necrosis was superficial and partial, repair was rapid; if the necrosis was complete, regeneration was confined to the slight extension of a unicellular layer over the damaged area.

In the anal canal portions of the mucous and of the cutaneous lining were resected with minimum trauma to the underlying layers. In these experiments the extent of the regeneration of the mucous lining was the same as in the colon and contrasted with the

complete regeneration of the cutaneous lining of the anal canal. To explain this difference it is suggested that the cells that spread from the mucosa over the denuded area are equivalent to mucous-secreting cells and have no capacity to form new glands.

**The vascular density of the mucous membrane of the lesser curvature and pars pylorica of the human stomach compared with that of the greater curvature.**

By F. S. A. DORAN. *All Saints' Hospital, Bromsgrove, Worcs.*

Chronic peptic ulceration of the stomach is confined to the lesser curvature. This fact suggests that anatomical peculiarities may exist in this part of the stomach. The blood supply of the lesser curvature has been suspect for over 30 years. Reeves (1920) thought the vessels in the sub-mucosa were smaller and more tortuous. Barlow (1951) has stated that there is no difference in the blood supply in the gastric mucosa of the lesser curvature.

The present study, using an experimental and statistical method (Fisher, R. A., 1946, 1947), seeks to demonstrate that there is a definite tendency for the vascular density of the lesser curvature to be lower than that of the greater curvature.

**The development of Houston's valves in the human embryo and foetus.**

By P. H. S. SILVER. *Middlesex Hospital Medical School, London*

The variation in the histological appearance of Houston's valves in the adult human rectum suggested the study of these structures in the embryo and foetus. Fifteen embryos and foetuses have been examined.

By the 10th week definite infolding of the rectal wall may be seen which includes not only the mucous membrane but also some of the circular muscle fibres. By the 14th week the rectum has become sinuous and the longitudinal muscle coat is incorporated into the developing valves, which lie approximately at right angles to the long axis of the gut.

During the later stages, Houston's valves typically contain all the layers of the rectal wall including the whole of the longitudinal muscle coat and a core of connective tissue which is continuous with the perirectal connective tissue. Both these layers extend almost to the free edge of the valve, but are separated from it by a thickened layer of circular muscle fibres. Although variation was seen in the position and number of valves, the histological picture of individual valves was uniform.

**The hands of Insectivora.** By R. WHEELER HAINES. *University of Sheffield*

The hands of the tree shrews *Tupaia tana* and *Pltilocercus lowii* have been studied by serial section and graphical reconstruction, and compared with those of a typical shrew *Crocidura* and a macroscelid *Elephantulus*. The tree shrews show arboreal specialization in the ridging of the palmar pads and in the origin of the contrahentes muscles from raphe similar to those found in the clasping hands of opossums and monkeys. In all the insectivores examined the interossei take origin from the carpal bones by a peculiar fibrous sheet, sometimes provided with sesamoids over the bases of the metacarpals. The muscles have no attachment to the metacarpals though their bellies may bulge dorsally into the interosseous spaces. The evidence from the hand supports the retention of the Insectivora as a natural group, with the tree shrews included as a branch specialized for arboreal life.

**The lung form in Artiodactyla.** By D. BROWN.

*Charing Cross Hospital Medical School, London*

Gelatine injected specimens and bronchial corrosion casts of the lungs of members of the order Artiodactyla have been prepared, including at least one from each of the three suborders. Each species so far examined has shown an asymmetrical right upper lobe arising directly from the trachea proximal to its bifurcation.

The lungs of *Lama glama* representing the suborder Tylopoda consist superficially of a single unlobed lung on each side, and resemble more the lungs of the Equidae.

The lungs of the remaining suborders Suiformes and Ruminantia show a more typical configuration. In the former the lungs are of approximately equal size but in the Ruminantia the right lobe is enlarged in the three families so far examined. In the Cervidae and Bovidae the lobe forms at its apex a large pyramid placed almost centrally over the trachea. In the Giraffidae the right upper lobe is considerably larger than the left and is deeply grooved by the azygos vein in the three specimens so far examined.

The development of the right upper lobe has been followed in four pig embryos of 9, 10, 11 and 13 mm., c.r. length and its migration from the tracheal bifurcation demonstrated.

#### **Studies on the anatomy of the nose.** By V. E. NEGUS.

*Ferens Institute of the Middlesex Hospital, London*

As part of a comprehensive examination of the nose and sinuses of vertebrates examples are given of three methods employed:

(a) *Anatomical.* Transparent tinted reconstructions of the nose of a human foetus at the fifth month, and of the nose of a rabbit (*Lepus cuniculus*).

(b) *Histological.* The mucosa has been examined in serial sections. The epithelium, usually described as respiratory, has been found in the rabbit to be of two types; on the septum and in some other parts it is columnar and ciliated, but over the widely branched maxillo-turbinal it is very thin and stratified, with only two rows of cells. This delicate membrane is no doubt designed to allow of stretching when the underlying vascular spaces are filled and also to allow of free radiation of heat and transudation of fluid. The relative thicknesses of the three types of mucosa are: olfactory, 72-144 $\mu$ ; columnar ciliated, 21-64 $\mu$ ; stratified, 5.4-28 $\mu$ .

(c) *Experimental.* The degree of dilation possible in the nasal mucosa of a cat is seen in sections cut after injection of adrenalin and histamine, followed by intravital fixation of the animal, carried out by Mr J. C. Seymour of the Ferens Institute, Middlesex Hospital. The difference between the mucosa over the ethmo-turbinals and that covering the maxillo-turbinals is striking.

#### **The foot as a half-dome.** By J. MCKENZIE.

*University of Aberdeen*

The foot is usually described as having medial and lateral longitudinal arches and a transverse arch, although a few writers, notably Wood Jones, compare it to a half-dome, the weight being transmitted from the talus to the periphery of the foot, which forms roughly a half circle as seen in the footprint.

Three sound reasons why this latter method of regarding the foot should be more widely used are the facts:

(1) That the trabeculae within the tarsal bones radiate from the talus downwards and outwards to the periphery of the foot.

(2) That the functional arches in walking are not restricted to the longitudinal and transverse.

(3) That a better understanding of the deformities seen in flat-foot is obtained.

#### **Range of movement of the big toe.** By J. JOSEPH.

*Guy's Hospital Medical School, London*

The range of variation in movements at the metatarsophalangeal and interphalangeal joints of the big toe in fifty males, in approximately three equal age groups, has been investigated by means of lateral X-ray films. In the normal plantigrade position there is, as a rule, some dorsiflexion at both the joints, but fairly often there is plantar flexion at the interphalangeal joint, sometimes associated with a more marked dorsiflexion at the metatarsophalangeal joint. There is a very wide variation between individuals in the range of movement. There is much more dorsiflexion at the metatarsophalangeal joint than plantar flexion. The opposite is the case at the interphalangeal joint. There are no significant



differences between the right and left sides in both movements at both joints. Plantar flexion is significantly decreased at both joints in the older age group but dorsiflexion is not.

Passive dorsiflexion at both joints has been measured also and a negative relationship found between active and passive dorsiflexion, i.e. the greater the angle of active dorsiflexion the smaller is the angle of added passive dorsiflexion.

**Characteristics of the East African vertebral column.** By D. B. ALLBROOK,  
*Makerere College Medical School, Kampala, Uganda*

Observations have been made on two hundred East African vertebral columns in the anthropological collection of the Department of Anatomy. Numerical variations were more common than in any race previously examined. The modal number of twenty-four presacral vertebrae occurred in 85 %, whilst 13 % had twenty-five and 2 % and twenty-three presacral vertebrae. The cervical spinous processes were bifid in 54 %; they were variable in form and unlike those found in European skeletons. The superior articular facets at the thoraco-lumbar transition became thoracic in type at T12 and L1 with equal frequency. Asymmetry of these facets occurred in 24.3 % of columns, the highest incidence so far reported. The bone component of the lumbar curve was assessed by measuring the anterior and posterior lengths of the vertebral bodies. The point of contraflexure was between L3 and L4. In male columns there was a significant increase of length between the ages of 20 and 45 years. Bony exostoses occurred in a high proportion of vertebrae, young as well as old. In the thoracic region bony spurs occurred on the superior (and occasionally the inferior) margins of the laminae. Dissections showed these to be ossifications in the ligamenta flava. They were found in 65 % of skeletons. Bony spurs, suggestive of ossification in the anterior longitudinal ligament were found in 21 % of skeletons.

**Some observations on the intra-epidermal sweat ducts.**

By N. CAUNA. *King's College, Newcastle upon Tyne*

The intra-epidermal sweat ducts have been studied in the human hairless digital skin and compared with the hairy skin as well as the skin from the digits of the Rhesus monkey, the prehensile tail of the *Lagothrix* monkey and the pads of the domestic cat.

The course of the sweat ducts has been studied by means of a series of wax-plate reconstructions. These show that the sweat ducts are passages coiled in a clockwise spiral. In the human digital epidermis they are more coiled and voluminous than in the comparative material examined, and the capacity of a digital sweat duct is approximately equal to the entire secretory portion of the sweat gland. The spiral course increases the length of the sweat duct approximately four times as compared with a straight vertical duct.

It is suggested that the intra-epidermal sweat ducts act as containers from which a constant and even impregnation of the stratum corneum with sweat is possible.

In the hairless digital skin sweat passages open along the midline of the papillary ridges with the result that each ridge is bisected longitudinally by a series of spiral perforations. This arrangement increases flexibility between the two halves of the ridge and may facilitate the mechanism of transmission of touch stimuli to the receptors.

There is no evidence to support the suggestion (Krieg, 1924) that the course of the sweat duct indicates the spiral pathway which epidermal cells describe in their movement towards the surface.

**Alkaline phosphatase activity in the developing teeth of the rat.**

By N. B. B. SYMONS. *University of St Andrews*

This was studied from the 18th day of foetal life to 4 days after birth, using the simultaneous coupling azo dye method. Sodium  $\alpha$ - and  $\beta$ -naphthyl phosphates were employed with the diazonium salts of 4-chloro-*o*-anisidine and 5-chloro-*o*-toluidine. The material was either fixed in cold formalin and frozen sections cut, or in cold absolute alcohol and embedded

in paraffin wax; the material was not decalcified. Some sections were treated by the Gomori calcium-cobalt method for comparison.

Previous investigations, using the Gomori method, give varying accounts of the detailed distribution of the enzyme. The particulate distribution of the dye and the absence of nuclear staining in the coupling azo dye method give a clear picture. Best results were obtained using sodium  $\alpha$ -naphthyl phosphate and especially with frozen sections; though the thinner paraffin sections obtainable and their greater definition when counterstained with haematoxylin made them valuable as a control.

The site of greatest enzyme activity was in the stratum intermedium, followed in intensity by a band of pulp tissue immediately deep to the odontoblasts; the external enamel epithelium showed about an equal degree of activity while the odontoblasts showed somewhat less. The only enzyme activity shown by the ameloblasts was at their basal ends, i.e. adjacent to the stratum intermedium. The general pulp tissue showed only very slight staining, except near the tip of the incisor of the 4-day-old rat where the ameloblasts have shortened and the entire cytoplasm of these cells shows heavy staining.

**The development of the ductus venosus in man.** By A. D. DICKSON.  
*The Queen's University, Belfast*

Between the 5 and 12 mm. stages the ductus venosus is transformed from a vessel joining the mid-points of the subhepatic and subdiaphragmatic anastomoses between the vitelline veins, into a channel connecting the umbilical vein to the inferior vena cava, the definitive condition. This transformation involves profound changes in the venous arrangements at the two ends of the ductus, which have been analysed in a series of embryos of intermediate crown-rump length. The main conclusions are that at the lower end the left half of the subhepatic anastomosis rotates into line with, and becomes the terminal part of, the umbilical vein, while at the upper end the right half of the subdiaphragmatic anastomosis is almost entirely absorbed into the common hepatic vein (terminal cava).

**A basis for further classification of malformations within the groups so-called cor biloculare and cor triloculare biatriatum.** By F. P. REAGAN. *University of Birmingham*

Suggested subtypes are: (a) that in which the primitive embryonic 'sinus venosus' has remained widely communicating or confluent with the 'primitive (= common) atrium', in which cases, maximum survival is about six postnatal weeks; (b) cases in which the left half ('horn') of 'primitive sinus venosus' and the left half of 'primitive atrium' have become partitioned, the original sort of communication of the 'left horn' with the 'right horn' of sinus venosus being maintained, a condition under which maximum survival is apparently one year; (c) the basal or dorso-sinistral portion of the right (sinus) horn has undergone torsions whereby the 'left horn' (dextral end) of the coronary sinus looks to have been dragged so that it gained (normal) entry into a sinistro-sternal compartment of the definitive right atrium, the maximum survival of that ostensible condition being to late teen-age.

Anomalies (a) and (b) may have in common also: persistence of V. cava superior sinistra; complete [a] or incomplete [b] persistence of infracardial, thoracic vena cava inferior; greatly hypertrophied left horn (coronary sinus usually mistaken for common atrium) receiving the intrinsic veins of the heart.

Type (b), exemplified by a specimen personally studied, is supplemented by brief considerations of Type (a) as gleaned from early nineteenth-century literature.

Apparently the majority of cases of supposed cor biloculare do not conform to either of these types.

**Pattern of cartilage canals in the upper tibial epiphysis of foetal sheep.**

By C. LEVENE. *The Queen's University, Belfast*

A series of foetal sheep from 65 to 200 mm. C.R. length was injected with indian ink in plasma, and the knee region dissected out and cleared. Examination of the cleared specimens, by transmitted light with the stereoscopic microscope, revealed a number of vascular canals in the upper tibial epiphysis. These were broadly but consistently divisible into ventro-medial, ventro-lateral and dorsal groups which sprang from a common stem just above and behind the tibial tubercle. This system of canals was supplied by a single artery, a branch of the upper end of the popliteal artery, which ran around the medial side of the joint close to the medial ligament. In addition to this main system, a number of smaller independent canals entered from all parts of the margin of the epiphysis but did not penetrate very far. No anastomoses between the groups of canals were seen, but connexions with the metaphyseal blood vessels were established in the later stages across the growth cartilage. The significance of these findings and the problems they arouse were discussed.

**Observations on the early development of the mammalian maxilla.**

By A. D. DIXON. *The Queen's University, Belfast*

The early development of the maxilla was studied in man, sheep, pig, rabbit, cat, and rat embryos. In all cases the bone developed from a single centre of ossification appearing constantly in the angle between the infraorbital and anterior superior dental branches of the maxillary nerve. To this basic 'neural' or body element, frontal, zygomatic, palatal, external and internal alveolar processes were added. Areas of hypertrophic secondary cartilage were found transiently in the zygomatic process of the rat and man, and also in the palatal suture and alveolar margin of the rat.

In all species examined early developmental stages were remarkably constant, both in respect to general form and in the relations of the maxilla to the maxillary nerve branches, the nasal capsule and the dental lamina. Similar constancy of nerve, cartilage and dental relationships in early mandibular development has been noted by other workers, suggesting that these relationships are not accidental but may indicate inductor activity.

**The results of mating following (1) unilateral tubal ligation and (2) unilateral ovariectomy in rats. By A. YOUNG. *University of Glasgow***

The left Fallopian tube was ligated in forty-four young adult, virgin rats (group A), and the right ovary was removed in twenty-four similar animals (group B). All animals of groups A and B were mated 4 weeks after treatment. In group A, thirty-seven animals had normal pregnancies in the right horn of the uterus (non-ligated side). Of these thirty-seven, twelve animals had swellings in the left horn of the uterus (ligated side). In these twelve, the swellings in the left horn were sectioned and examined histologically and the findings were as follows: One had three normal pregnancies and in the others the swellings were deciduomata. Twenty-three animals from group B had normal pregnancies in the left horn of the uterus (non-operated side). There was a small swelling in the right horn of the uterus in one animal, histologically identified as a deciduoma. The significance of these results was discussed.



APRIL 1954

An ordinary meeting of the Society for the Session 1953-4 was held on Friday, 23 April 1954 in the Department of Anatomy, King's College, Strand, London, W.C. 2. The President (Prof. W. J. HAMILTON) was in the Chair.

The following are the authors' abstracts of the papers read.

**Hormone control of lipid in the reproductive tract of the female guinea-pig.**

By T. NICOL and R. S. SNELL. *King's College, London*

The distribution of lipid in the reproductive tract was studied in 104 female guinea-pigs. The results were as follows:

(1) Lipoid first appears in the epithelium of the distal part of the uterine body 60-70 days after birth.

(2) During the oestrous cycle lipid was present in the epithelium of the uterine horns and in that of the distal uterine body during the period of activity of the corpus luteum, but was practically absent at the time of implantation. The lipid appeared to be an index of cell activity. In the vagina, lipid was only present at the time of oestrus and can be demonstrated in the cells of the vaginal smear. The appearances suggested that the shedding of the epithelium at oestrus was a process of fatty degeneration.

(3) The lipid appearances in the uterus were reproduced experimentally in fifteen ovariectomized animals by injections of oestrogen and progesterone.

(4) After ovariectomy lipid disappeared from the uterine horns in about 4 days, but remained in the distal part of the uterine body until about the eleventh day after operation.

(5) Large lipid-containing reticulo-endothelial cells were present in the stratum compactum throughout the oestrous cycle and were most numerous about the eleventh day. After ovariectomy there appeared to be a cyclic distribution of these cells independent of the ovaries.

**The roots and spinal origin of the phrenic nerve in the rhesus monkey.**

By G. A. G. MITCHELL, E. P. SAMUEL and R. WARWICK. *University of Manchester*

The motor fibres of the phrenic nerve are reputedly derived from a distinct group of nerve cells in the ventral grey column which extends through several segments of the cervical spinal cord. The clinical and topographical evidence quoted in support of this 'phrenic nucleus' is scanty and inconclusive; experimental evidence, in primates at least, appears to be lacking.

The retrograde changes following division of the phrenic nerve in monkeys have been therefore investigated. Initial interruptions, effected in the neck, show that all available accounts of the arrangement of the roots of this nerve in the monkey are inadequate. To ensure complete division of these roots at operation, 23 dissections have been carried out, and from these it is apparent that the phrenic nerve receives a contribution from the nerve to the subclavius in at least 50 % of cases, and that its fibres could be derived from the third to sixth cervical segments.

Division of the phrenic nerve has been carried out in twenty-five animals, both in the neck and at various thoracic levels. A preliminary examination of serial sections of the spinal cords from fifteen of these show that unquestionable retrograde degeneration can be produced in this instance, and that these effects are confined to a small number of motor cells, which frequently form a topographical group in the central grey column of several cervical segments.

**The conjoined twins of Kano.** By I. AIRD. *Post Graduate Medical School of London*  
(introduced by W. J. HAMILTON)

A film (prepared by S. Schofield Productions, Ltd.) was presented showing the clinical investigation and operative treatment of the conjoined twins born in Kano, Nigeria, in

July 1953. The investigation which showed the exact nature of the pattern of conjunction was described. The steps of the operation were demonstrated and the cause of death of one of the twins discussed. A short account was added of the anatomy of two further sets of conjoined twins recently born dead in England.

**Myelination of the axons to Pacinian corpuscles.** By T. A. QUILLIAM  
and M. SATO. *University College, London*

The histology of the Pacinian corpuscle has been described on many occasions. Hitherto little attention has been paid to the position of the nodes of Ranvier along its fibre or to the distribution of myelin within the corpuscle itself, factors of importance for analysis of the method of functioning of this receptor.

Twenty-six corpuscles with their nerve fibres have been dissected out from the mesentery of the small intestine of cats and stained either by osmic acid (0.5 %) in isotonic saline or by a modified gold chlorine technique. In the proximal part of the corpuscle the fibre is tortuous and its outline irregular. In the central core of the corpuscle, however, the fibre is narrower, straight, fairly uniform in calibre, and lightly stained. The fibre terminates at a little distance from the distal extremity of the corpuscle as two or more separate swellings.

A node of Ranvier is invariably present on the axon just proximal to its point of entry into the corpuscle. The convoluted segment of the axon inside the corpuscle often shows a discontinuity of its myelin sheath which seems to be morphologically similar to a node of Ranvier. The first internode situated completely outside the corpuscle varies in length from 144 to 496  $\mu$ , whilst the diameter of the fibres in these regions ranges from 4.1 to 6.9  $\mu$  (twelve specimens). There is some evidence to suggest that central internodes are longer than peripheral ones.

The lengths of the corpuscles vary between 0.46 and 1.55 mm. In general, the larger corpuscles possess fibres characterized by longer internode distances.

**The anomalous richness of the lung in lymph vessels.** By  
CHARLES C. MACKLIN. *University of Western Ontario, Canada*

The lymph vascular supply of the lung seems greater than is warranted by the bulk and metabolic activity of the tissue served, and this suggests a special lymph source. Since all respiratory membranes are moist, the alveolar walls must be so. Macklin (1954, *Lancet*) submitted that the alveolar surface area (100 sq.m. in man) is wetted by a special pneumocyte-transmitted fluid, and that residue from the 400 cu.cm. of water which it relinquishes daily to the atmosphere is eliminated mainly by lymph vessels. This variable overflow from the shallow but expansive alveolar lake was sensed by Sikorsky (1870, *Cent. med. Wiss.*), Klein (1874, *Proc. Roy. Soc.*) and others, but their conceptions of stomata were rejected. In man and other mammals areas of alveolar epithelium and underlying lymphatic tissue recall the histomorphology of tonsils and suggest that they are thoroughfares for redundant alveolar fluid containing stray particles. Such minutiae are phagocytized here, though some may pass to hilar lymph nodes and even farther. These air-tight alveolar outlets (approaches to lymph vessels) have been found in walls of alveolated bronchioles and contiguous to pulmonary arterioles and venules, subpleura and interlobular septa always intimately related to lymph vessels. These sites, in dust-breathing animals, are marked by particle deposits, often spectacular. They may admit living bacteria and cells, and thus become involved in disease processes, as tubercles and cancers. Plethora of alveolar fluid may condition alveolar oedema, while paucity may be associated with medical emphysema.

**Structures associated with the completion of the enamel matrix.**  
By N. B. B. SYMONS. *University of St Andrews, Dundee*

In the incisor teeth of the rat at the region where production of the enamel matrix is ceasing a number of globular structures are constantly found in the ameloblast layer and

stratum intermedium. These have been interpreted previously as formed by fusion of granules of organic calcium compound no longer absorbed by the complete matrix, or as eleiden globules. With routine stains these structures all appear similar. With pyronin-methyl green staining, however, four types are seen: pyroninophil globules often near the distal end of the ameloblasts, irregular nodules staining deeply green, similarly shaped nodules staining purplish-pink, and a few large pyroninophil globules containing one or more of the nodular structures. Sections treated with ribonuclease before pyronin-methyl green staining show only the nodular bodies. With Feulgen's method irregular nodules are stained. With the P.A.S. method all types stain pink.

These findings suggest that at the point associated with the completion of the enamel matrix and the beginning of enamel maturation the ameloblasts undergo a profound change involving even the degeneration of some cells. This is supported by the vacuolation constantly found in this area. The irregular nodules may be nuclear remnants; unlike the globules they are not found towards the distal end of the ameloblasts. The globules may be the coalescence of secretory products including protein and polysaccharide, no longer required for matrix production.

**Cytological observations on surgically removed pituitary glands.** By C. L. FOSTER and R. R. WILSON. *St Mary's Hospital Medical School, London*

Observations were made on the anterior lobes of two human pituitary glands which had been surgically removed. Portions were immediately fixed in formol-saline, Helly's fluid, Champy's fluid and Baker's formol-calcium-cadmium solution. Frozen and paraffin sections were studied by ordinary and phase contrast microscopy. It was found that the appearances closely resembled those seen in post-mortem pituitary glands fixed two and three hours after death.

**Observations on secretory activity in the oviduct of the ewe.**

By R. HADEK. *University of Glasgow Veterinary School*

Oviducts of sixty sheep from all phases of the sexual cycle were studied for the presence of morphological and histochemical changes. It was found that the height of the epithelial cells increased during pro-oestrus and oestrus, was unchanged during met-oestrus and decreased during di- and an-oestrus. The presence of secretory and ciliated cells was a constant feature in the epithelium whereas 'rod' cells were only visible during di-oestrus. Evidence was found that the latter were degenerating secretory cells.

With histochemical methods an increase in the ribonucleic acid content of the ciliated and secretory cells was observed during pro-oestrus and oestrus. In the same periods secretory granules accumulated in the secretory cells in which in addition alkaline (glycero-) phosphatase could be demonstrated. Both the secretory material and alkaline phosphatase were found in the lumen of the oviduct during met-oestrus. The secretory granules were P.A.S. positive and with different successive histochemical tests were found to contain mucopolysaccharide.

## JULY 1954

The Summer Meeting of the Society for the Session 1953-4 was held on Friday and Saturday, 2 and 3 July 1954, in the University of Cambridge. After the first morning session owing to the large number of communications the meeting was divided into two sections, working concurrently, the one in the Lecture Theatre of the Anatomy School and the other in the Lecture Theatre of the Department of Pathology. The President (Prof. W. J. HAMILTON), Professors J. J. PRITCHARD, J. S. BAXTER and Dr G. J. ROMANES occupied the Chair at the various sessions.

The following are the authors' abstracts of the papers read.



**The later history of the primitive streak in the sheep embryo.**By J. D. BOYD. *University of Cambridge*

Notwithstanding the extensive literature on the developmental history of the mammalian primitive streak there is little detailed information on its retrogression and final disappearance. Access to a source of carefully aged sheep embryos has enabled a collection of a series of sections to be made which include at least one specimen from each 24 hr. period from the 15th to the 28th day inclusive. From a study of this material the stages in the retrogression and disappearance of the primitive streak were described. Observations on mitoses and on cell degeneration in the hinder end of the embryos during these stages were included.

**Nucleic acids in the cord and dorsal root ganglia of the developing chick.**By A. F. W. HUGHES. *University of Cambridge*

Ester wax sections through the cord and ganglia at various levels have been photographed with an ultra-violet microscope at 2537 Å, a wave-length corresponding to the peak in absorption of the nucleic acids. Measurements have been made of the thickness of the sections (about  $2\mu$ ) and of the density of nuclear and cytoplasmic images in the negatives. The results have been expressed as optical density per micron thickness of section. The cytoplasm of the ependymal layer and of the ganglionic neurones, and also the mitotic figures of the ependymal zone have so far been studied in this way.

There is a peak in cytoplasmic density at four days of incubation, followed by a minimum during the two successive days. Similar features are seen in the curve given by Novikoff and Potter for ribonucleic acid in the whole embryo, expressed as mg. RNA per unit of dry weight. During the fourth day also, the density of absorbing material in the mitotic figures increases.

Some possible correlations with the differentiation of the nervous system may be traced. By the end of the fourth day, most of the main peripheral nerve trunks have appeared, which are thus developed during a period of relatively high cytoplasmic nucleic acid. Again, at 5-6 days, pycnotic nuclei are common in the ventral horn of the cord and dorsal root ganglia at non-limb levels, as Hamburger and Levi-Montalcini have shown. At this time the cytoplasmic density of nucleic acid in the neurone is at a minimum.

**Function of the jaws in mammals.** By C. C. D. SHUTE.*University of Cambridge*

An analysis has been made of the jaws of mammals in relation to the stresses incurred at the temporo-mandibular joint during the act of biting against resistance. In Carnivora the condyles are transversely expanded in response to a torque set up by the temporalis and masseter muscles. The medial side of the glenoid cavity is commonly produced postero-inferiorly to resist the contraction of temporalis, and the lateral side supero-anteriorly to resist masseter. The degree to which these lips are developed can be correlated with skull and jaw proportions which determine the direction in which the forces act. The following closely related genera are compared: the European badger and the African ratel, *Melivora*; *Hyæna* and the aard-wolf, *Proteles*; the northern fox and the African big-eared fox, *Otocyon*.

It is argued that the high mandibular ramus of herbivores is related, not, as American workers claim, to tooth occlusion, but to the function of the masseter muscle. The temporo-mandibular joint is not elevated in the giant panda, *Ailuropus*, in which the temporalis appears to be dominant. In this animal the dental arcades are extended backwards as an adaptation to a herbivorous diet; in consequence the lower glenoid lip is particularly prominent. Analysis of the stresses at the joint in, for example, the bush-baby, *Galago*, shows that they are mainly horizontal. This is associated with the flat glenoid cavity typical of herbivores, and facilitates protraction and side-to-side movements.

The problem is raised of the apparently herbivorous character of the jaws of elephant shrews (Macroscelididae).

**Vascular patterns in the spinal cord of the rat.** By D. H. M. WOOLLAM and J. W. MILLEN. *University of Cambridge*

By injecting into the ascending aorta of the rat a red dispersion which passed through the arterioles into the capillaries and veins and following this with the injection of a blue dispersion, which did not enter the capillary bed, the vascular patterns of the spinal cord were completely outlined and the arteries were easily recognizable from the veins. Sixteen cords were examined. After photographing the vessels on the surface of the cord, sections were cut of the various regions in the transverse, coronal and sagittal planes.

The anterior spinal artery of the rat closely resembles that of man in its method of formation, course and distribution. It is formed above by the junction of branches from both vertebral arteries and extends the length of the cord, being augmented by a few major radicular arteries. In the upper lumbar region the largest of these radicular arteries, which corresponds to the great spinal artery of Adamkiewicz in man, divides into ascending and descending branches which replaced the anterior spinal artery in this part of the cord. In the thoracic region, the anterior spinal artery becomes extremely slender and almost peters out. The significance of the radicular contribution to the anterior spinal artery is discussed.

**Morphological observations on the bones of a pig on a low plane of nutrition from birth.** By A. B. MORRISON and R. A. McCANCE. *Department of Experimental Medicine, Medical Research Council, University of Cambridge*

One of a pair of female litter-mate piglets was fed from the age of 7 weeks on a greatly reduced ration of a normal diet, while its fellow was fed unlimited amounts of the same ration. The animal which was reared on a high plane of nutrition increased in weight from 24 to 350 lb. during the 24 weeks of the experiment, whereas its undernourished litter-mate increased to only 54 lb. during the same time. There was a notable difference in body form, which was most evident in the differently shaped heads. When the animals were killed at the end of 7 months the bones of the undernourished animal were lighter and of finer texture and not so well marked by the muscles; their epiphyseal cartilages were narrower and smoother and there was great thinning of the cortical bone. There seemed to be no defective calcification and no lines of arrested growth were present.

The skull of the undernourished animal was different from the normal in external form; the cranial cavity had the same dimensions as the normal but the bones of the vault were very thin compared to the thick vault of the normal. The distance from the occipital condyles to the premaxillae in proportion to the distance from the condyles to the frontal sinus was greater in the normal animal (25.5:10.5) than in the undernourished animal (18.5:9) which suggests that the fore-shortening of the head in the undernourished animal was largely in the facial bones.

**Development of limb abnormalities in *Xenopus* after treatment with a nitrogen-mustard.** By P. TSCHUMI. *University of Cambridge* (introduced by J. D. BOYD)

After the local application of a nitrogen-mustard solution to the limb-buds of *Xenopus* larvae, limbs with various abnormalities develop.

After treatment with very dilute solutions and as a consequence of a reduction in the blastema size syndactylous and 4-, 3- (or less) toed legs develop. Within the reduced blastema, however, the primordia of the toes, etc. are of about normal size. Consequently the first ones to appear occupy relatively too much space and the later appearing toes are suppressed by 'physiological competition'.

Other abnormalities (polydactyly, abnormal or missing proximal parts, etc.) develop after treatment with higher concentrations of the antimitotic drug and subsequent destruction of most of the bud-mesenchyme. Such 'empty' buds may later produce one or several isolated areas of blastema which independently give rise to more or less abnormal limb-parts.

**Experimental hypovitaminosis-A in relation to hydrocephalus in the rabbit.** By J. W. MILLEN and D. H. M. WOOLLAM (*University of Cambridge*) and G. E. LAMMING (*University of Nottingham*)

For many years it has been recognized that a deficiency of vitamin A in the diets of experimental and farm animals is associated with the subsequent appearance of lesions of the nervous system. In an extensive literature going back to 1916, xerophthalmia, blindness, muscular inco-ordination, paresis and death have been described in animals which were deprived of vitamin A. These reports embrace observations on a wide range of animals including cattle, pigs, puppies, rabbits, rats, hens and ducks. For the most part, however, the accounts deal with animals which were subjected to the deficiency of vitamin A after birth.

In a recent paper (Millen, Wollam & Lamming, *Lancet*, 2, 1953) a preliminary account was given of experimental work on the relationship between vitamin A deficiency in female rabbits and the development of hydrocephalus in their young after birth. Further investigation has shown that, when the dams have been fed on a diet deficient in vitamin A for long periods before mating, a hydrocephalic condition may already be established in the offspring at birth. Of fifty-one young examined from twelve dams, forty-seven were found to have hydrocephalus. These findings are discussed in the light of current views on the production of hydrocephalus and the part played by hypovitaminosis-A in the aetiology of lesions of the nervous system.

**Anterior rhizotomy of preganglionic fibres in man.**

By P. A. G. MONRO. *University of Cambridge*

Observations on anterior rhizotomies in the upper thoracic (T1, T2, and T3) and in the lumbo-sacral (T12-S2) regions in man have shown that sympathetic activity in the limbs is inhibited initially (diminished sweating and decrease in vasomotor tone) but that within three weeks or so, a great deal of this activity has returned. This suggests that preganglionic fibres not ordinarily synapsing with the ganglion cells which supply the limbs, have been able to form functional connexions with these cells, which have been deprived of their original preganglionic supply. It is suggested that they do so by sending out new collaterals to these ganglion cells as they course farther along the sympathetic chain towards those cells which they have always supplied. This process appears to be one of 'functional re-organization', as has been described in the cat by Geohegan & Aidar (*Proc. Soc. exp. Biol. N.Y.*, 50, 1942).

**Studies on the maintenance *in vitro* of embryonic and neonatal rat and mouse adrenals.** By J. D. LEVER. *University of Cambridge*

In the early part of this century various workers attempted the maintenance *in vitro* of the mammalian embryonic, neonatal and adult adrenal cortex. In this work small fragments (0.5 mm. thick) of the gland were cultured on a plasma clot medium. Poor cell survival was reported in adult glands; all surviving tissue became progressively de-differentiated with loss of lipid inclusions. Levenstein, Gordon & Charipper (*Proc. Soc. exp. Biol.*, N.Y., 46, 1941), employing a plasma clot medium with serum-tyrode supernatant, contended that unlike the thyroid and pituitary, fragments of adrenal are not well maintained *in vitro*.

A method is described for whole gland adrenal culture in which the organ is floated on a fluid medium. In the 18- to 21-day rat embryo adrenal the majority of mitoses are in the peripheral cortex and cell survival in culture is confined to this peripheral zone. In contrast to the neonatal rat adrenal, which is not well maintained *in vitro*, the corresponding mouse gland shows regions of complete cortical and partial medullary survival after 3 days of culture. Work is proceeding on the effects of ACTH and other substances on the growth and behaviour of rat and mouse adrenocortical cells *in vitro*.



**A fossil lemuroid skull from East Africa.**By W. E. LE GROS CLARK. *University of Oxford*

An exceptionally well-preserved lemuroid skull, almost complete except for the mandible, has recently been discovered in the Early Miocene deposits on Rusinga Island in the Kavirondo Gulf of Lake Victoria. It displays several primitive features and also shows an interesting combination of characters found among recent genera either in the Lorisinae or the Galaginae. The skull is probably referable to the genus *Progalago* (MacInnes), but it differs markedly from the other (much less complete) lemuroid skull which was derived from the same deposits and previously described by Le Gros Clark & Thomas (1952).

**The organization within neurons.** By R. W. G. WYCKOFF (*Science Attaché, American Embassy, London*) and J. Z. YOUNG (*University College, London*)

Electron microscopy of ultra-thin sections of neurons of the spinal cord of rabbits shows a very dense, seemingly vacuolated, spherical nucleolus and nearby one or more irregular masses of somewhat less opaque material, having a net-like structure. The rest of the nuclear material is a much less dense network of short straight threads enclosing clear spaces. The threads are frequently paired and carry granules at the nodes. The nuclear membrane appears for the most part as a continuous line with regular thickenings, but it is sometimes interrupted and what may be oblique cuts show a reticulum with opaque paired trabeculae.

The cytoplasm contains great quantities of opaque material distributed in the classical pattern of Nissl substance. Between these masses are channels containing many mitochondria and large opaque spheres (? Golgi bodies, lipochondria). Impregnation of these channels would produce a classical Golgi reticulum.

The Nissl substance is in many places a network of tangential and radial trabeculae. Both of these are frequently paired. There are signs of continuity or organization between the Nissl substance and the nucleus. Shells of Nissl substance lie close to the nuclear surface but often there is a relatively clear perinuclear region with mitochondria and lipochondria.

In the dendrites the Nissl substance often appears organized into a system of tangential trabeculae, frequently paired and studded with radially distributed granules.

The mitochondria are rod-like and crossed by opaque bands with lighter spaces between. They have a thin surface membrane and some longitudinal striation. There are forms that suggest possible transitions between the Nissl substance and mitochondria.

**The nerve cell surface.** By R. W. G. WYCKOFF (*Science Attaché, American Embassy, London*) and J. Z. YOUNG (*University College, London*)

As seen by electron microscopy of ultra-thin sections the outer border of the cell body and dendrites of a ventral horn cell is not bordered by any tissue space but is occupied by a series of spheres containing many mitochondria. These lie between the protoplasm of the neuron and that of the glia. Sometimes they appear separated from both by thin membranes but since all the materials are in full contact, decisions about boundaries are very difficult.

Presynaptic nerve fibres lie surrounded by glia protoplasm. They have a distinct and highly opaque sheath and axoplasm of low opacity. Their outer surface may be in direct contact with the cell membrane or with that of one of the surface spheres. Structures resembling boutons are rare. It is uncertain whether the surface spheres are continuous with presynaptic nerve fibres.

Capillaries are also surrounded by glia protoplasm and all interchanges of the neuron must take place through this which is very rich in mitochondria.

The surface spheres can easily be seen in the light microscope after fixation in formalin, postchroming, carbowax embedding and staining with modified Weigert or silver methods developed by Mr K. C. Richardson, which stain the mitochondrial material.

**Observations on the autonomic outflow to pelvic ganglia and viscera in human embryos.** By P. CALABRISI. *University of Cambridge*

A study of silver impregnated human embryos shows that the adult pattern of autonomic supply to the pelvis is established very early. Graphic reconstruction enabled the origin and course of the nervi erigentes to be traced. In general, the findings confirm those of Browne (*Anat. Rec.* 116, 1953) but the larger number of embryos available allows of a more detailed account.

**An histological investigation of six baroreceptor areas of the right common carotid artery in the cat.** By J. BOSS and J. H. GREEN. *Middlesex Hospital Medical School, London*

The following are the six baroreceptor areas of the right common carotid artery in the cat:

Area	Nerve		Relation to arterial branching	Original descriptions	
	Cranial N.	Branch		Activity	Histology
1	X	R. aortic n.	At origin from innominate a.	Green (1954)	Nonidez (1935)
1a	X	R. aortic n.	No branch	Green (1954)	Present studies
2	X	R. aortic n.	No branch	Green (1954)	Present studies
3	X	R. aortic n.	No branch	Green (1954)	Present studies
Common carotid area	X	R. common carotid n.	At origin of ramus muscularis dorsalis and supr. thyroid a.	Green (1953)	Present studies
Carotid sinus	IX	R. sinus n.	At terminal bifurcation	Hering (1923); Bronk & Stella (1932)	De Castro (1928)

After the detection of baroreceptor activity by electro-neurography, lengths of artery were removed, fixed, impregnated with silver by Ranson's method and serially sectioned. There were characteristic structures, confined to pressure-sensitive regions in the adventitia of each area. Histological control showed the structures to be nervous and they were in anatomical continuity with myelinated fibres which were aggregated into nerves identifiable with those from which baroreceptor impulses had been recorded during life.

Even in areas without arterial branching the media and adventitia were modified in the presence of nervous structures. In area 1 and the carotid sinus these structures were related to the bifurcation in a characteristic way and sometimes entered the media.

The interpretation of the apparent structure of the nervous elements, their relation to arterial branches and their embryology, were discussed.

**Nuclei of IX, X and XI complex of cranial nerves.**

By G. A. G. MITCHELL and R. WARWICK. *University of Manchester*

These nuclei have been investigated by several workers in rabbits, cats and dogs with variable results. They have been studied in Primates by sectioning the IX, X and XI nerves at many levels (the sites will be indicated) in forty-seven macaque monkeys, and by subsequent examination of the brain stems, upper cords and samples of the nerves proximal and distal to the sites of division. To date, sections from thirty-five brain stems have been examined. So much material is involved that in this communication consideration will be limited to the dorsal vagal nucleus, but it may be mentioned that changes have been noted also in the ambiguous, solitary tract and intercalated nuclei. The chief points demonstrated are: the reputed grouping of small cells cranially and caudally and of large cells in the middle of the nucleus is not particularly evident in *m. rhesus*; three types of cells, and not two as commonly stated, are distinguishable; the

consistent occurrence of chromatolytic changes in the nucleus following section of the vagus at every level; evidence of partial bilateral representation, when sections are made below the level of the lung roots, indicating intermixture in the oesophageal plexus; a suggestion that representation is inverted; and proof that the middle part, the 'nucleus cardiacus nervi vagi', is not concerned entirely with cardiac innervation.

**Departure of a colloid from the subarachnoid space.** By F. HOWARTH and E. R. A. COOPER. *Universities of Cambridge and Manchester*

The fate of a radioactive colloidal palladium of known particle size was studied after subarachnoid injection in a hundred cats. The colloid was introduced via the lowest reaches of the sacral subarachnoid space or by an improved technique of cisternal injection. The concentration of the colloid fell in the cerebrospinal fluid and rose in the blood. The influence upon this process of the pressure and route of introduction and posture of the animal was examined. Finally the rate of passage of this colloid from the subarachnoid space to the blood was investigated with simultaneous recordings of arterial, sagittal sinus and cerebrospinal fluid pressures.

It was shown that there was no significant circulation of the cerebrospinal fluid in the spinal subarachnoid space of the anaesthetized cat, and that this colloid passed from this space without ascent to the cerebral subarachnoid space.

Attempts to demonstrate histologically the precise route of egress of the colloid from the subarachnoid space were unsuccessful, since it was difficult to detect these small particles in blood and tissues.

**Reinvestigation of Weed's evidence for the route of drainage of the cerebrospinal fluid.** By E. R. A. COOPER and F. HOWARTH. *Universities of Manchester and Cambridge*

It has been shown previously that certain naturally occurring ions and a spinal anaesthetic pass from the spinal subarachnoid space into the local venous drainage. Colloidal palladium appears to behave similarly. Weed (1914) stated that the spinal subarachnoid space is drained via the lymphatics. His work has been repeated.

The iron ammonium citrate and potassium ferrocyanide mixture in isotonic solution formulated by Weed has been used. The method of introduction into the subarachnoid space has been either that used by Weed or one of the methods described in the previous communication. In spite of Weed's assertion to the contrary, this mixture appeared to be a convulsant. It has been found that at pressures of introduction within the physiological range, the mixture did not fill the cerebral subarachnoid space even after periods up to five hours. Histological evidence is presented for the direct passage of this foreign substance into the small blood vessels within the theca. Weed's evidence for the functional significance of the arachnoid villus is discussed.

**A study of the nerve cells of the grey matter of the lumbo-sacral spinal cord in man.** By W. J. W. SHARRARD. *Institute of Orthopaedics, London*

Using projection microscopy at  $\times 100$  magnification, reconstructions of the nerve cell content of the grey matter from total serial sections of the lumbo-sacral cord have been made. The cytoarchitectonic structure is shown to be a laminar one. A topographical analysis of the motor cells in the anterior horn shows twelve main columns; their position and extent are compared with the analyses of previous workers. The sizes of the cells in any given cell column are characteristic of that column. The mean number of cells in each motor cell column in each spinal segment is given, the total for the whole lumbo-sacral cord being 24,600 in each anterior horn.

A plan is given of the localization of function in the motor cell columns, derived from a study, by the same method, of anterior horns in four cases of acute anterior poliomyelitis dying 5, 22, 36 and 72 months after the onset of the disease.



**Remyelination in regenerating sural nerves.**By T. A. QUILLIAM. *University College, London*

Quantitative studies of transverse sections of normal sural nerves of rabbits have shown that the myelinated fibres they contain lie between 1 and  $16\mu$  in total diameter (Weigert-Pal technique) and that they produce no myelinated branches for distances of up to 7 cm. The diameter size frequency distributions of these fibres are unimodal in character with a peak in the  $2-4\mu$  or  $4-6\mu$  diameter size group (Quilliam, Abstracts 5th International Congress of Anatomy, Oxford, 1950).

The absence of fibre tapering can be conveniently demonstrated in any of these specimens by comparison of the total cross sectional area of all the myelinated fibres in a proximal section with that in a distal section.

Using similar techniques, no changes can be detected in those myelinated fibres lying proximal to the level of the lesion in sural nerves regenerated for periods of between 25 and 300 days after a crush operation.

Parity between the numbers of myelinated fibres immediately distal to a crush site and those found proximally is first attained 55 days after operation, but this state of equality is not achieved at all peripheral levels examined until 200 days after a crush. At no time does the number of myelinated fibres distally in any one specimen exceed that found proximally. The largest myelinated fibres immediately peripheral to a crush never attain the diameter of the largest ones proximally, but this discrepancy becomes less marked as the period allowed for regeneration increases. A progressive reduction in calibre of the largest myelinated fibres is noted as sections situated at successively more distal levels peripheral to a lesion are examined. This phenomenon is also most marked in early material.

Between the 25th and 35th days of regeneration the 'wave front' of myelination proceeds peripherally at a mean rate of 4 mm. per day.

**The clearance of  $^{24}\text{Na}$  from a mammalian nerve trunk.** BY M. J. BLUNT and K. STRATTON. *Royal Free Hospital School of Medicine, London*

The rate of clearance of radioactive sodium injected into the sciatic nerve trunk of rabbits has been used as a means of studying the effects of various experimental interruptions of the blood supply to the nerve.

The tibial division of the sciatic nerve was injected with approximately 0.005 ml. of Krebs-Ringer solution containing about  $2\mu\text{c}$  of  $^{24}\text{Na}$ . The activity over the injection site was recorded, so that the rates of clearance could be calculated. Standard experimental procedures were employed to test the immediate effects of interruption of blood supply to the nerve.

It has been found that the effective circulation to the sciatic nerve depends in acute experiments more upon the regional branches of supply than upon the longitudinal intrinsic vascular plexuses of the nerve. Groups of animals in which the nerve was insulated from contact with the surrounding tissue fluid and other groups in which the nerve was allowed to lie in its muscle bed were compared: it has been found that the initial clearance rates were higher in the latter group and the effect of vascular interruption was less marked. The significance of these findings is discussed.

**Cell production in the liver of the developing chick.** By J. PERRY. *University of Cambridge* (introduced by A. F. HUGHES)

Many workers have emphasized the extremely low mitotic activity in normal adult liver tissue and have speculated on the possibility that 'cryptomitosis' would account for the growth of the liver after visible mitotic phenomena have ceased. The work in progress deals with the increase in total numbers and with the mitotic index of the chick liver at various stages of incubation from six to nineteen days.

Cell suspensions were prepared from a known weight of chick liver by the trypsin digest method described by Moscona & Moscona (*J. Anat., Lond.*, **86**, 1952). Complete digestion of the intercellular matrix was accomplished in half an hour and a homogeneous suspension was assured by mechanical mixing. These suspensions contained both vascular and hepatic cells which were easily distinguished except at very early stages of incubation. Cell counts were made on a haemocytometer slide and the total hepatic cell count estimated from the total wet weight of each liver.

The mitotic index was counted from liver sections ( $5\mu$ ) after similar periods of incubation. Fixation was carried out with Flemming's fluid and nuclear staining with Heidenhain's haematoxylin.

Preliminary results suggest that the rate of hepatic cell formation is very rapid in the initial stages of development, dropping to a lower level later, but that there is still an increase in total cell numbers during the latter period of incubation when the mitotic index has fallen to a very low figure.

**Observations on the development of the intra-cranial rete in the sheep.** By K. BALANKURA. *University of Cambridge*

From a study of a series of sheep embryos it has been found that in the 30 mm. stage the para-hypophyseal part of the internal carotid artery begins to break up into smaller vessels. Simultaneously the maxillary artery sends branches through the foramen ovale and the superior orbital fissure into the cranial cavity. These branches of the maxillary artery anastomose with those of the internal carotid, forming the intra-cranial rete. Once the anastomoses are established the internal carotid artery caudal to that level gradually diminishes in size and finally becomes a fibrous cord or disappears completely.

**The gubernaculum testis of certain ungulates.** By K. M. BACKHOUSE, H. BUTLER and D. H. WOODHEAD. *St Bartholomew's Hospital Medical College, London*

Investigation of the gubernaculum testis in pig, ox and sheep fetuses (150–400 mm. greatest length) has shown that macroscopically the gubernaculum testis is an elongated, ovoid mass of jelly-like tissue, closely resembling in appearance Wharton's jelly: it is attached to the caudal pole of the testis with the cauda epididymis embedded in its anterior aspect. The gubernaculum testis is suspended by a short mesentery continuous above with the mesorchium. The gubernacular mesentery is attached to the posterior abdominal wall and later to the posterior wall of the processus vaginalis: as testicular descent proceeds, attachment becomes limited to the latter.

Microscopically, the gubernaculum testis consists of a solid mass of mesenchyme. The blind tip of the processus vaginalis consists of a mass of similar mesenchymatous tissue, continuous, *via* the base of the gubernacular mesentery, with the substance of the gubernaculum testis.

The cremaster muscle is a long, flattened bundle which runs caudalwards behind the processus vaginalis. Its fibres are continuous above with those of the internal oblique muscle and below fan out to be inserted into the posterior wall of the processus vaginalis.

Precisely similar structures have been found in human fetuses after the testis has passed through the inguinal canal. In none of the species examined could the gubernaculum testis be described as a fibro-muscular cord or strand, the descriptive terms of current usage. Furthermore, in all the specimens of all the species examined, the processus vaginalis and its contents could easily be lifted out of the scrotum and nowhere was there evidence of any anchoring effect customarily ascribed to 'tails of Lockwood'.

**Observations on the vitelline veins and yolk-sac of 11 mm. C.R. twin and other human embryos.** By W. R. M. MORTON. *Queen's University, Belfast*

Unichorionic bin-amniotic twin embryos of 11 mm. C.R. length, excellently preserved and serially sectioned in the transverse and sagittal planes, have been examined along with other human embryos. A large left vitelline vein, traversing the intra-embryonic

coelom independent of the mesentery of the mid-gut (in which lie the superior mesenteric vein and the omphalo-mesenteric artery) has been found in these and in single 11, 16 and 22 mm. c.r. human embryos, but not in 4, 5 and 8 or 12 mm. c.r. embryos. In the 11 mm. embryos the outer end of the vitelline vein leaves the mesenchymal investment common to the vitello-intestinal duct and vitelline vessels at the apex of the gut loop, passes backwards and upwards in the upper part of the umbilical opening close to the body wall, and then runs without a mesentery to its point of union with the superior mesenteric vein at the left side of the duodenal loop to form the omphalo-mesenteric vein. The vitelline artery on the other hand is a continuation of the omphalo-mesenteric artery from the apical region of the gut loop and, unlike the vein, remains in the mesentery of the mid-gut. A similar condition has been recorded in the cat (Dexter, *Amer. J. Anat.* 1, 1902).

The wall of the definitive yolk-sac in embryos ranging from 4 mm. to 47 mm. c.r. length has also been examined. Endodermal thickenings and vesicles are features of the early stages but these disappear later. The conspicuous columnar, coelomic mesoderm cells covering the yolk-sac persist longer than the endodermal vesicles. By the 47 mm. stage however, the yolk-sac shows marked degenerative changes in all elements.

### **The ductus venosus in the pig.**

By A. D. DICKSON. *Queen's University, Belfast*

The ductus venosus, which, in a wide range of animals, connects the left end of the sinus intermedius to the left hepatic vein, is generally believed to disappear at an early stage of development in the pig. The examination of a series of specimens, ranging from 14 to 210 mm. crown-rump length, shows that the ductus does not disappear in its entirety. Its caudal portion is replaced by a plexus, while its cranial portion remains as the vein draining the plexus.

The plexus lies in the liver close to the floor of the oesophageal groove. It is supplied with umbilical vein blood by a number of branches which arise from the left end of the sinus intermedius and travel cranially. These branches spread out and come to lie at intervals around the periphery of the plexus. From them the blood passes centripetally through the finer ramifications of the plexus, where it comes into intimate relationship with hepatic epithelial cells. It traverses vessels little larger than sinusoids, separated from the hepatic cells only by endothelium and a layer of connective tissue of the same order of thickness as that found in the wall of the sinusoids themselves. The vessels collecting the blood from the plexus lie centrally in it. They are radicles of the single vein which opens into the left hepatic vein.

### **The regeneration of the uterine epithelium at the placental site in post-partum rats.** By J. G. WARBRICK. *University of Glasgow*

Rats were killed at six-hourly intervals from 0 to 48 hr. after littering. Portions of uteri were fixed in either Bouin's or Rossman's fluid and serial sections were prepared. Those fixed in Bouin's fluid were stained with haematoxylin and eosin, and those fixed in Rossman's fluid were treated to show the distribution of either glycogen or ribonucleic acid. The placental site was re-epithelialized within 48 hr. by a spread of cells from the epithelium at the margin of the site. No glycogen was found in the epithelial cells at any stage. Ribonucleic acid was relatively reduced in amount in epithelium that was spreading over the placental site. The significance of these findings was discussed.

### **Observations on the secretory activity in the uterus of the sheep.**

By R. HADEK. *University of Glasgow Veterinary School*

The uteri of sixty non-pregnant sheep from all phases of the sexual cycle, including anoestrus, were studied for the presence of morphological and histochemical changes.

It was found that the height of epithelial cells in the uterus of the sheep increased during oestrus and metoestrus and reached the maximum height during early dioestrus when it



appeared pseudo-stratified. Thereafter it decreased in height. The epithelium which lines the uterine glands followed the increase of the uterine epithelium but on a lower scale. The cells lining the uterine lumen and those lining the glands appeared to be similar during pro-oestrus. The presence of a large number of leucocytes in the epithelium was noted during early and late di-oestrus. With histochemical methods an accumulation of minute lipid globules was noted at the base of the epithelial and glandular cells during oestrus and metoestrus. During dioestrus the globules appeared also in the distal part of the cells. A P.A.S. positive material was noted in the proximal part of the epithelial and glandular cells during met- and early dioestrus, which together with the lipid appeared in the uterine and glandular lumen during early dioestrus. It was found to be a mucoprotein. The presence of alkaline phosphatase was a regular feature in the epithelial and glandular cells during met- and dioestrus. The reacting material occupied a narrow zone immediately beneath the free surface of the cells during early dioestrus and was never seen in the lumen of the uterus or glands. Inorganic iron was found in the glandular cells only. It formed minute granules which appeared in the distal half of the cells during oestrus and metoestrus. During dioestrus their number increased. A few granules were occasionally found in the glandular lumen. Mucopolysaccharide only appeared in the uterine lumen during met- and dioestrus and its origin was traced to oviduct secretion.

#### **The vascular pattern of the rat uterus in pregnancy.**

By A. YOUNG. *University of Glasgow*

The changes in the uterine vascular pattern of the Albino Wistar rat have been followed throughout pregnancy in a series of 138 animals. In sixty of these, one horn was rendered sterile prior to mating to provide a control vascular pattern. A double injection technique of the vascular system with radio-opaque media or latex was used as previously described (Young, 1952). The development of the maternal placental circulation was described and certain findings which differ from the conventional description were indicated. Variations were found in the degree of vascular filling in different pregnancy sites in the same animal and the sterile horn differed in this respect from the areas between pregnancies in a normal horn. These observations were discussed.

#### **The production of instantaneous footprints.** By C. H. BARNETT.

*St Thomas's Hospital Medical School, London*

A new type of pedograph, made up of a large number of transparent perspex rods mounted vertically on 'Sorbo' rubber, has been used to record the changing distribution of pressure beneath the foot during walking. It is inset within a wooden platform, illuminated from one side and photographed from the other, at 30 frames per sec., while a subject walks upon it. The rods are marked in such a way as to produce on each photograph an indication of the foot outline and the distribution of pressure.

A short film was shown to illustrate some of the results obtained.

#### **The relation of the nutrient artery to the growing end of the femur.**

By P. A. RING. *Charing Cross Hospital Medical School, London*

The nutrient canal of the human femur is directed away from the distal end, whilst that of the rabbit femur passes towards it. In each case the distal end grows more rapidly than the proximal and the point at which the nutrient canal would intersect the centre of the shaft of the bone indicates the site of the primary centre of ossification. In the foetal femur both of man and rabbit the nutrient canal originally passes distally and the change of direction during growth can be attributed to the varying modes of growth of periosteum and bone. The site and direction of the nutrient canal in the adult appears to be determined by the mode of entry of the nutrient artery at the onset of ossification.

**Respiratory air conditioning and thermoregulation.**By P. COLE. *University of Manchester*

From a study of comparative anatomy, Scott, (*J. Anat., Lond.*, **87**, 1953) suggests that the nasal turbinate structures are concerned with thermoregulation. Confirmatory evidence obtained from a study of air conditioning within the respiratory passages is now presented.

Man has simple turbinates and yet during nasal breathing at rest inspiratory air of arctic condition is warmed to 31° C. and almost saturated with water before it reaches the larynx. In temperate conditions inspiratory air temperature in the trachea is about 35° C. and it is as high during oral as during nasal breathing.

Mucosal blood-borne heat may be less essential for warming inspiratory air than is commonly assumed, since a glass tube containing moist blotting paper extracts sufficient heat from expiratory air to warm and moisten inspiratory air as effectively as does the nose. The heat and water content of nasal expired air varies very considerably. Both heat and water are recovered as air passes out from the lungs and the recovery increases with the coldness and dryness of the environment. In man breathing air of arctic condition about half the heat and water added to inspiratory air is recovered during expiration. Heating the body surface remote from the respiratory passages increases turbinate mucosal blood flow and thus increases heat and water loss in expired air.

An animal with complex turbinates such as a dog recovers in cold conditions more, and in hot conditions less, heat and water from expiratory air than does man.

It is suggested that a function of the turbinates is to conserve heat and water. The conservation is adjusted to the needs of the body by adaptative changes in mucosal blood flow similar to those which occur in the skin of the extremities, and is of considerable importance in well-insulated animals.

**A method of estimating skull capacity from radiological diameters.**By I. L. MACKINNON. *King's College, London*

The true capacities of fifty-two dry adult skulls were obtained by measuring the volume of lead shot required to fill them.

Various diameters of the cranial cavities were then measured in centimetres from antero-posterior and lateral radiographs of the skulls and the optimum combination for estimating capacities from them was calculated.

The diameters used were (i) The maximum internal length (*L*). (ii) The maximum internal breadth (*W*). (iii) The longest measurement from the top of the external auditory meatus to the vault (*H*). (iv) The maximum distance from the deep aspect of the bregma to the cerebellar fossa (*B*). The optimum combination was

$$\{\frac{1}{2}(L \times H \times W) + \frac{1}{2}(L \times B \times W) \times 0.51\},$$

and was subject to an average error of 2.2 % and a maximum error of 6.18 %.

**Structural changes induced by ischaemia in the kidney of the rabbit.**By R. G. BURWELL. *University of Leeds* (introduced by A. DURWARD)

Rabbits were subjected to temporary renal ischaemia by clamping the renal artery for periods of 1.5–4 hr., and the resulting structural changes were studied after periods of reflow from 6 hr. to 4 weeks.

In some animals (31 %) structural changes were absent or minimal, but in 62 % of animals, structural changes were distributed throughout the renal parenchyma and were maximal in the descending segment of the proximal tubule, where, after six hours reflow, almost complete necrosis had occurred. In some tubules, a few cells survived at the junction with the thin segment of Henle's loop. These surviving cells became actively phagocytic, ingested the adjacent necrotic debris and swelled so as to occlude the lumen. Their cytoplasm was vacuolated and many of them showed numerous hyaline droplets. Histochemical studies suggested that there may be two distinct morphological manifestations

of protein reabsorption by the cells, a conclusion in keeping with the findings of other workers.

These findings appeared to shed light upon Selye's (Selye & Stone, *J. Urol.* (Baltimore), **56**, 1946; Selye, *Trans. J. Macy Jr. Foundation*, New York, 8-9 Jan. 1948) interpretation of 'the endocrine kidney', after subjecting rats to chronic partial renal ischaemia, he found the development of active cellular proliferation within the terminal portion of the proximal convolution and concluded that these cells were the source of the renal pressor substance. The writer is inclined to the view that Selye was in fact describing cells which had undertaken an active phase of protein reabsorption, secondary to some tubular necrosis.

**The effect of anoxia on the bone marrow.** By E. H. BATTEN, W. J. GALL, G. HALLEY, R. S. HARRIS, A. F. ROGERS and J. M. YOFFEY. *University of Bristol*

A quantitative technique has been applied to the study of the cellular changes in the bone marrow occurring during anoxia. Fifteen control experiments were performed in Berne, and twenty-eight at the Hochalpine Forschungsstation, Jungfraujoch, at a height of just over 11,300 ft.

The marrow responds to the anoxia by producing increased numbers of red cells, but there is a latent period of 3-5 days before these are discharged into the blood in sufficient numbers to increase the peripheral red cell count. Pooling the experiments done during the first five days at the Jungfrau and comparing them with the controls, the peripheral blood showed a mean reticulocyte increase from 80,000 to 163,000 per c.mm. ( $tC - E = 3.19$ ), the marrow erythroid cells rose from 190,000 to 345,000 ( $t = 3.1$ ), and the marrow lymphocytes fell from 213,000 to 107,000 ( $t = 3.81$ ).

Furthermore, associated with the fall in the number of marrow lymphocytes there were interesting qualitative changes, including what appeared to be numerous transitions between small lymphocytes and blast cells.

**The phase contrast microscope and alkaline phosphatase.**  
By N. M. HANCOX and EVELYN NICHOLAS. *University of Liverpool*

Changes have recently been described (*Nature, Lond.*, **173**, 1954) in the phase-contrast appearance of sections when mounted direct after incubation in the Gomori substrate bath used for the histochemical demonstration of alkaline phosphatase. The striated border of duodenal epithelial cells first becomes invisible, then phase-negative, and, subsequently, impregnated with fine crystals. Both phase changes and crystals are abolished after brief exposure to dilute acids. Gypsum crystals grow out from the incubated border when sections are mounted in melted glycerine jelly acidified with  $H_2SO_4$ . These facts indicate that calcium is certainly present in the border after incubation, probably as  $Ca_3(PO_4)_2$  as would be expected. Representative illustrations of these phenomena are shown.

Comparison has been made of the apparent distribution of enzyme in tissue sections, as seen after the classical Gomori-cobalt sulphide technique and by means of direct phase contrast microscopy. Sections from freshly prepared paraffin blocks and from similar blocks after four months' storage have been used and we have compared results obtained with different phosphate esters as substrate. The results indicate that the phase method provides a more complete and quantitative representation of enzyme in the striated border. The cobalt sulphide technique provides misleading results (often seriously so), tending to over-emphasize the sites of minimal activity and to suppress the maximal.

**Histochemical observations on the neurohypophysis in dog and cat, with reference to the relationship between neurosecretory material and posterior lobe hormone.** By J. C. SLOPER. *Institute of Pathology, London Hospital* (introduced by R. J. HARRISON)

Following Bargmann's demonstration of neurosecretory material (N.S.M.) throughout the neurohypophysis, it has been widely held that the hormones of this region are formed



in the hypothalamus rather than in the infundibular lobe of the pituitary (Scharrer & Scharrer, 1954). Attempts have been made to corroborate this theory of hypothalamic neurosecretion in the dog and cat, by studying first the relative distribution of various esterases and phosphatases in the hypothalamus as compared with the infundibular lobe: and second, the tinctorial and histochemical properties of N.S.M. These investigations cast doubt on the complex and variable glycolipoprotein composition of N.S.M. as suggested by Schiebler (1952) and suggest that it is more probably a protein, akin to posterior lobe hormone.

Thus, first, the relative distribution of esterases and acid-phosphatase in supra-optic neurones and infundibular lobe was compatible with hypothalamic neurosecretion: unfixed frozen sections alone were used. Second, N.S.M. was demonstrated in paraffin sections by chrome-haematoxylin and aldehyde-fuchsin (with a longer preoxidation than recommended by Halmi, 1951). The tinctorial specificity of N.S.M. was confirmed by a new stain, phosphotungstic-acid congo red (Swettenham & Sloper, 1954, unpublished). The properties of these three stains suggested that N.S.M. was a protein. This was established histochemically; in particular it was shown that N.S.M. could be demonstrated in alcohol-fixed tissue if the resultant paraffin sections were floated on Bouin's fluid instead of on water: that N.S.M. was removed by trypsin: and that in paraffin sections sudanophil lipid and P.A.S. positive carbohydrate were scanty throughout the neurohypophysis, and often unrelated to N.S.M. in neurones. This protein was shown probably rich in cystine by virtue of its reactions with alkaline tetrazolium (Pearse 1953), and, after thioglycollate-treatment, with potassium ferricyanide (Hardy 1953) and dihydroxydinaphthyldisulphide (Barnett & Seligman 1954). The first two showed the exact distribution of N.S.M., thus indicating the uniform composition of N.S.M. whether in its intracellular or extracellular distribution. It is suggested that this cystine-rich protein represents the oxytocic and vasopressor octapeptides synthesized by du Vigneaud *et al.* (1953).

#### **Further observations on the blood supply of the rabbit's ear.**

By BRUNO ROSSATTI. *University of Cambridge* (introduced by J. D. BOYD)

The vascular arrangement of the rabbit's ear has been re-examined by means of the neoprene latex injection technique and the usual histological methods. The arterial and venous patterns of this organ have been studied in thirty-five animals of different ages. Both these vascular systems possess an annular anastomotic disposition associated with which are a large number of arterio-venous anastomoses. A detailed account of the topography, number and shape of these arterio-venous shunts is given. Finally, some observations on the experimental regeneration of arterio-venous anastomoses are presented.

#### **Observations on vascular perfusion and fixation of the lungs.**

By BERNARD TOWERS. *University of Cambridge*

A perfusion-technique has been devised which permits direct observations to be made on rat lungs maintained in a condition simulating full physiological expansion. A heart-lung preparation, with a perfusion-cannula tied into the pulmonary trunk, is suspended from a tracheal cannula which passes through the lid of an air-tight transparent chamber. A controlled partial vacuum produces a standard degree of pulmonary expansion, and a preliminary saline-perfusion is followed by either fixative, coloured saline, or some other injection-medium. If either the saline wash-out or the subsequent perfusate is cold, the extent of tissue-penetration is very variable. This is readily seen with a coloured perfusate, when sometimes discrete patches of colour 2-3 mm. in diameter can be observed on the pleural surface, demarcating the areas of distribution of vessels supplying secondary pulmonary lobules. Increase of intra-vascular pressure (within physiological limits) does not necessarily affect the extent of the penetration, and even after thirty minutes' perfusion there may still be areas left uncoloured by the dye. If, however, both the saline wash-out and the subsequent perfusate have been warmed in a water-bath, immediate and complete penetration results at pressures less than 10 mm. of mercury.

The technique provides standard conditions for histological fixation, and accurate measurements can therefore be made of various microscopic dimensions in the lungs of experimental animals. Slides are shown of the histological pictures obtained.

**The vascularization of the adrenal gland in the rhesus monkey.**

By R. G. HARRISON and C. W. ASLING. *University of Liverpool*,

The two adrenals differ in the sources of origin and number of arteries supplying the gland. The left adrenal is supplied by about twenty arteries derived from a trunk arising from the coeliac artery (which divides into inferior phrenic and adrenolumbar arteries), the aorta and left renal artery. The right adrenal has a more profuse arterial supply from both superficial and deep sources. The superficial arterial supply provides some twenty arteries derived from an inferior phrenic artery (which takes origin from the aorta near the renal artery) and right renal artery, while the deep arterial supply, derived entirely from the aorta, contributes about eighteen arteries. A minor variation can occur in the arterial supply of the right adrenal. Both adrenals are drained by two veins which pass into the inferior vena cava on the right and renal vein on the left. The intraglandular pattern of vessels is similar to that in other mammals, arteriae medullae also being present.

Interruption of individual adrenal arteries produces focal necrosis in the adrenal cortex when examined 5, 7 and 21 days later. The necrotic area involves only the zona fasciculata 21 days after operation. Focal necrosis may not occur after interruption of adrenal arteries at a distance from the gland on the right side because of a collateral circulation between adrenal arteries effected through renal capsular vessels.

**The results of interruption of the inferior mesenteric artery and its branches in the rat.** By J. L. BRAITHWAITE. *University of Liverpool*

Observations on the distribution of the inferior mesenteric artery and its branches have been carried out in six normal rats and in forty animals undergoing operation. Four types of operation have been performed:

- (1) Interruption of the stem of the inferior mesenteric artery and its subdivisions.
- (2) Interruption of the marginal artery of the gut.
- (3) Interruption of the branches of the marginal artery (arteriae rectae) to the gut wall.
- (4) A combination of the above three.

The animals were sacrificed at periods varying from twenty-four hours to five months after operation and injected with Micropaque (Damancy & Co.). Following dissection, a segment of gut was removed and an arteriograph taken; selected specimens were studied histologically. All animals except four (following type four operation) survived.

The results demonstrate the great potentiality of the collateral circulation, in particular the importance of the intrinsic anastomoses between branches of individual arteriae rectae on the gut wall. The inferior mesenteric artery can be interrupted at its origin and the marginal artery along any part of its course without complication. If, however, a series of six or more consecutive branches of the marginal artery are interrupted necrotic changes are produced in the gut wall. Removal of the marginal artery together with the branches arising from it can be carried out only to a limited degree.

**Regional histochemical differences in the epididymis of the adult rat.**

By R. B. MANEELY. *University of Liverpool*

Evidence was presented of regional differences in alkaline glycono-phosphatase activity in the epididymis as shown by the Gomori technique. Sections of the caput and cauda were incubated together for progressive periods of time. The results showed that a positive reaction could be elicited earlier in the caput than in the cauda epididymis, and indicated differences in the quantity, or degree of activity of the enzyme present in these parts.

Evidence was also presented of regional quantitative differences in polysaccharide complexes shown by the periodic acid-Schiff reaction. An epithelial secretory cycle was

known to occur throughout the epididymis but the experimental results indicated that its rhythm may be quicker in the caput than in the cauda, the amount of P.A.S.—positive material being maximal in the lower portion of the caput.

**Effects of sub-toxic doses of propylthiouracil on body growth and on some representative viscera of young rats.** By C. W. ASLING and R. G. HARRISON.  
*University of Liverpool*

Groups of female albino rats (ten per group, averaging 60 g. body weight at onset) received thiouracil in subtoxic doses for 29 days. The dosage was precise, being injected as 6-*n*-propylthiouracil (in slightly alkaline solution), 0.3 mg. per day for 15 days and 0.6 mg. per day for the remainder. Effects on body growth, and on heart, kidneys, thyroid and adrenals (ponderal and histologic) were reported. Goitrogenicity was satisfactorily demonstrated at this dosage, thyroid weights being increased more than 250 % (to 32 mg. as against 9 mg. on controls), and colloid goitre appearing histologically. However, there was no impairment of gain in body weight or length. Weight gain averaged 1.9 g./day, as in controls. Lengths averaged 295 mm. in treated rats and 302 mm. in controls. Adrenal weight and cortical histology remained essentially normal, as did the heart and kidneys. Some delay in opening of the vagina suggested impaired sexual maturation.

In some groups, treatment with desoxycorticosterone acetate (DOCA, 2 mg. twice daily) or with highly purified growth hormone (250  $\mu$ g. twice daily) was instituted after 5 days (thiouracil injections being continued). Some aspects of the response to these hormones deserved mention. Among them were the effect of DOCA on impairing body growth and adrenal weight and structure, and increasing kidney weight. The most noteworthy effect of growth hormone, apart from enhanced body growth, was a substantial further increase in the already marked thyroid hypertrophy.

**Cell proliferation in the gall bladder of the guinea-pig after ligation of the common bile duct.** By F. JACOBY. *University College, Cardiff*

The enormous mitotic activity of the gall bladder epithelium which follows ligation of the common bile duct has previously been reported (Jacoby, *J. Physiol.* **119**, 1953). A further analysis of the material shows that, apart from the epithelium, practically all other cell types which are capable of division, are also stimulated to proliferation. The mitotic activity of these cell types (serosal and vascular endothelium, smooth muscle and connective tissue cells) are surveyed; also some preliminary results obtained with gall bladder cultures are reported, which have been undertaken in an attempt to elucidate the mechanism underlying this proliferative activity. The problem of this mechanism is discussed.

**The changes in the regional lymph nodes following skin homografting in the rabbit.** By R. J. SCOTHORNE and I. A. MCGREGOR. *University of Glasgow*

A single full thickness homograft of skin (30 by 15 mm.) was placed on one ear of each of a number of rabbits. The animals were sacrificed at intervals before and after the time of destruction of the homografts. Sections of the regional lymph nodes from the operated and control sides were stained by the methyl-green-pyronin method.

Nodes from the operated side removed prior to the onset of homograft destruction show very large numbers of 'transitional cells' (Fagraeus, 1948). These are large cells, with a large pale nucleus, one or more prominent nucleoli and basophilic cytoplasm. They are thought to be intermediate stages between primitive reticulum cells and plasma cells. They are rare in nodes from unoperated controls and are much less numerous in the regional nodes of the unoperated ear of rabbits receiving homografts and in nodes from rabbits receiving skin autografts. In regional nodes removed after destruction of the homografts the number of transitional cells is much reduced.

These findings were discussed in relation to the problem of homograft destruction.



**Studies of cartilage autografts and homografts in rabbits.**

By M. B. L. CRAIGMYLE. *University of Glasgow*

Autografts and homografts of whole rib cartilage were implanted in rabbits intramuscularly and into the anterior chamber of the eye. They were removed at regular intervals up to 20 months in the case of the intramuscular grafts, and fifteen months in the intraocular series. The grafts were subsequently examined macroscopically and histochemically. The lipid droplets in the chondrocytes were examined using fettrot in propylene glycol as a solvent, and the glycogen content of the cells was demonstrated with the periodic acid-Schiff technique. The mucopolysaccharides of the matrix were examined using the Rinehart-Abul Haj modification of the Hale technique.

Each graft implanted intramuscularly was recovered and had retained its original form. During the 4 weeks following transplantation, there was sporadic loss of the glycogen and fat content of the chondrocytes which was more marked in the homografts. The loss was not seen after 28 days. Otherwise all autografts and homografts had survived and were histologically normal apart from some invasion by host fibrous tissue at each end.

In the intraocular series there was the same early change in lipid and glycogen content of the chondrocytes. Two homografts, one at 11 and one at 15 months were not recovered, although the remaining homografts had survived and showed the normal histology. All the autografts up to 15 months were normal.

The significance of these findings was discussed.

**The fate of frozen-stored cornea implanted subcutaneously in guinea-pigs. By**

P. BACSICH, G. PELC and G. WYBURN. *University of Glasgow and Radio-pathological Unit, M.R.C.*

Four guinea-pig corneae were frozen and stored by the method recommended by Parkes, for 4 weeks. The frozen corneal grafts and fresh corneal homografts were implanted subcutaneously into host guinea-pigs. All grafts were removed after 21 days, prepared and examined histologically. Sixteen hours before removal the host animals were given an injection of  $^{35}\text{S}$ . The fresh homografts showed the usual cyst formation and histological survival. Two of the stored grafts formed cysts smaller than those of the fresh graft but with apparently normal corneal tissue. The other two stored grafts were almost completely absorbed and the implantation site was only recognized by the presence of pigment cells. Autoradiographs of fresh and stored grafts were made and the uptake of  $^{35}\text{S}$  compared. These results were discussed.

**An improved microincineration technique for the demonstration of mineral elements in cells. By J. KRUSZYNSKI. *University of Liverpool***

The total distribution of minerals in tissues may be studied by microincineration technique. Some difficulties occur, however, when this method is applied to cells. Shrinkage of cells, dislocation and hydration of salts, and difficulties with illumination of the preparations under high power objectives are the main reasons why microincineration is regarded as a histochemical method rather than a cytochemical. With transmitted light the ash of cells and tissues cannot be seen, and dark-field illumination of incident light is generally employed. Phase-contrast illumination has proved superior, however, for cytological purposes, but this fact calls for further comment. The appearance of spodograms at various forms of illumination has been investigated by using preparations of different thicknesses, and it has been found that thin dried smears are the most suitable for cytochemical investigation. Spodograms of cells which have been completely flattened show nucleus, nucleolus and some cellular organoids. Shifting of minerals and hydration of salts are not pronounced. Phase-contrast is superior to dark-field and is essential for

cytological work with spodograms. Dark-field and incident light provide erroneous images of cells, due to: (i) reflexion from small mineral particles, or the masking of amorphous ash by reflexion from nearby larger deposits; (ii) hydrated particles reflecting more light than non-hydrated amorphous ash; (iii) the size of particles, when seen in dark-field illumination, appearing to be increased as a result of a halo effect.

**The occurrence of fat in the lining epithelium of the large intestine of the cat.** By B. F. MARTIN. *University College, Cardiff*

Previous histochemical studies on a number of species showed the lining epithelium of the large intestine to be rich in certain enzymes (alkaline phosphatase and 'lipase'). Although not by direct inference, the possibility was envisaged of sudanophilic material being present in this epithelium. This proved to be the case in the cat in at least half of the colons examined but not in the guinea-pig.

In an attempt to shed light on the significance of this observation, cats were subjected to the operation of ileostomy. The colon was emptied of its contents, a biopsy taken, and the cut proximal end closed, whilst the contents from the cannulated ileum were collected in a rubber balloon. After varying periods (up to 10 days), at a final operation prior to sacrifice of the animal, colon biopsies were taken both before and after the introduction of ileal contents into the lumen. These again showed, in several instances, the presence of sudanophilic droplets in the epithelium, but the amount of these was not influenced by the introduction of ileal contents.

The daily output of fat (petroleum ether extractable fraction) via the faeces before ileostomy was also compared with the daily output of this fraction via the ileal contents after operation. The faeces were found to contain a large excess of fat over that of the ileal contents.

The inference drawn from these results, namely, that the cat's large intestine excreted fat, linked up with results of older physiological studies.

**Histochemical changes in various tissues in scurvy.** By C. RUTH HILL and G. H. BOURNE. *London Hospital Medical College*

The exact role which ascorbic acid plays in the metabolism of the cell has not yet been determined. It is thought that a histochemical study of scorbutic tissues and organs might help to throw some light on this problem. Pair-fed guinea-pigs have been fed on rat cake supplemented with vitamins A and D and in the case of control animals with 10 mg. vitamin C daily.

Gomori's methods for esterase, lipase and acid phosphatase has been carried out. Scorbutic pancreas gives a stronger reaction than control pancreas for both esterase and lipase. Scorbutic liver gives a stronger reaction than control liver for acid phosphatase, the hepatic cells near the central vein being more intense than those at the periphery of the lobules. Seligman's succinic dehydrogenase method reveals a marked decrease in activity of scorbutic liver, kidney and skeletal muscle. The periodic acid-Schiff (PAS) test for polysaccharides has been carried out and formalin-lead acetate fixed tissues have been stained with toluidine blue for metachromasia. Hepatic cells of scorbutic liver show an increased basophilia with toluidin blue. Examination of costo-chondral junctions show that the edges of the calcified cartilage matrix of scorbutic pigs give a strong PAS-positive reaction. The most striking results have been obtained with scorbutic spleen where there is a great increase in the number of phagocytic cells of the red pulp which contain intensely PAS-positive droplets.

Whilst these preliminary results confirm that significant metabolic changes do occur in scorbutic tissues, their exact significance cannot at the moment be assessed.

**Sternal glands in the genus *Mandrillus*.** By W. C. OSMAN HILL.  
*Zoological Society of London*

Sternal glands have hitherto been reported in Primates only in the Pongidae and Cebidae. Ten years ago (*Nature, Lond.*, 1944, p. 199) reasons were given, based on behavioural features for suspecting the presence of a pectoral gland in the cercopithecoid genus *Mandrillus*. It is now possible to confirm this for both species of the genus. The glandular area occupies a more or less triangular field over the median part of the chest caudal to the nipples, and is clothed with modified hairs. Histologically three types of gland are present at different levels: (i) specialized sebaceous glands associated with follicles of the modified hairs, (ii) a stratum of enlarged sweat glands, (iii) a deep carpet of coil glands of peculiar type. The glands occur in both sexes.

**A technique for the orientation of serial histological sections.** By W. R. BURSTON and K. THURLEY. *University of Cambridge* (introduced by J. D. BOYD)

A fundamental requirement in any system of reconstruction from serial sections is some reliable method of orientation of the individual sections with respect to each other. Although many devices have been employed for this purpose, they tend on the one hand to be approximate and unreliable or, alternatively, accuracy is achieved at the expense of much money and time.

The method described combines considerable accuracy with speed and convenience in operation, so that it can be employed routinely in serial section cutting. A simple but accurate drilling machine has been designed to fit into the microtome knife-holder, and in this way it is possible to drill small holes (50-100 $\mu$ ) in the paraffin block which are at right angles to the plane of section. Previously prepared nerve fibres are inserted into these holes and sealed therein by a soft wax lute. The method has the advantage that no special embedding techniques are called for, the orientating nerve fibres are always mutually parallel and at right angles to the plane of section, and the fibres can be placed immediately adjacent to the part to be studied. (The fibres can be inserted into the specimen itself should this be thought desirable or necessary.)

The method is capable of extension to celloidin section cutting.

**Uptake of radio-active calcium by fracture callus.**

By J. J. PRITCHARD. *Queen's University, Belfast*

Radio-active calcium as calcium chloride was administered to adult rats either by subcutaneous injection or in the diet. Each animal received a total dose of 10 $\mu$ c. Subsequently radio-autographs of the skeleton were prepared. Radio-activity was demonstrable in the metaphyses and epiphyses and sub-periosteally soon after administration, and remained at these sites for long periods.

When the tibia was fractured one to four weeks before administration of radio-active calcium, the callus became intensely radio-active. On the other hand, if administration was stopped before fracture, then the callus showed little or no radio-activity.

It was concluded that the local and general skeleton was much less important as a normal source of calcium in fracture repair than generally believed, and that most of the calcium required came from the diet.

**Ossification of the neural arch.**

By J. MUTCH. *University of St Andrews*

The pattern of ossification in the neural arch appears to be essentially similar to that which is encountered in the diaphysis of a long bone. In each half of the neural arch the first calcification of cartilage occurs immediately subjacent to the perichondrium on the inner aspect of the interarticular region or 'isthmus'; in the fifth lumbar vertebra this is apparent at the 80 mm. stage. Thereafter, sub-perichondral bone is deposited on the inner



aspect of the isthmus in relation to the calcified cartilage, and this extends as the region of calcification increases. Subsequently there is a massive excavation of the calcified cartilage and only a few scattered islets remain on which bone is deposited within the isthmus.

This manner of ossification, which is common to all the lumbar vertebrae, is opposed to typical endochondral ossification which occurs in the epiphysis of a long bone. It appears to be inconsistent with the postulation that two centres may be found in each half of the neural arch; the condition of spondylolisthesis formerly was ascribed to a failure of union of two such centres.

#### **Time of appearance of fibular epiphyseal ossification centres.**

By F. G. ELLIS and J. JOSEPH. *Guy's Hospital Medical School, London*

The ossification of the human fibula is peculiar in that the centre for the proximal (growing) end appears later than that for the distal. Various mechanical reasons have been given in order to explain this peculiarity. Since the form and hence the function of the fibula vary considerably in different animals, the ossification of this bone has been studied in the rabbit, cat, guinea-pig, kangaroo, and pig and the literature searched for information on other animals. It has been found that in all the animals on which information could be obtained, the centre of ossification for the distal end appears before the centre for the proximal end. It is most unlikely therefore that the pattern of ossification of the human fibula is due to any special mechanical causes operating in man.

#### **Connexions of the internal vertebral venous plexus.**

By D. R. BOWSHER. *University of Liverpool*

Injection of radiopaque barium suspension into the azygos vein of the monkey, cat and rat fills the internal vertebral venous plexus. This consists mainly of two lateral longitudinal trunks lying in the extradural vertebral canal at the junction of pedicle and lamina. These trunks are interconnected in ladder fashion, and connect with extravertebral veins at every intervertebral foramen—chiefly the anterior sacral plexus, ascending lumbar veins, posterior intercostal veins, deep cervical and vertebral veins. The system is valveless.

The upper connexions of these lateral longitudinal trunks are with the inferior petrosal and occipital dural venous sinuses, and with the pterygoid venous plexus. These connexions have been caused to dilate (in the rat) by two-stage bilateral ligation of the internal jugular veins.

In the same animals there are direct connexions, particularly on the left side, between the internal vertebral venous system and the venous drainage of the adrenal gland, which do not involve the inferior vena cava or the renal veins.

Since the bronchial veins drain into the azygos system, it is suggested that these veins form a pathway for the metastasis of neoplasms and abscesses from the lungs, and that this accounts for their predilection to settle in the brain and adrenal glands. This may also explain the tendency for metastasis of tumours from the left adrenal gland, to the brain.

#### **A detailed study of the electric potentials from relaxed muscle and certain postural muscles of the leg and thigh.** By J. JOSEPH, A. NIGHTINGALE and P. L. WILLIAMS. *Guy's Hospital Medical School, London*

A circuit capable of extremely high amplification was used to record the electric potentials from the muscles of twelve young adult males. With the subject (*a*) fully relaxed upon a bed, and (*b*) in a defined symmetrical 'standing at ease' position, recordings were made from the soleus, gastrocnemius, tibialis anterior, quadriceps femoris and hamstring muscles, using surface electrodes. Recordings were also made from the skin overlying the tibia and patella, and recordings of amplifier noise were taken on each occasion.

The following results were obtained:

(1) Amplifier noise was remarkably constant from day to day and produced deflexions in the recordings of about  $1.5\mu\text{V}$ . peak to peak. On no occasion were deflexions of greater than  $2\mu\text{V}$ . recorded.

(2) Recordings from sixty relaxed muscles gave a constant picture which was similar to recordings obtained over the tibia and patella. All were distinctly greater than amplifier noise and frequent potentials in the  $3-5\mu\text{V}$ . range were seen in each case.

(3) In the standing at ease position all subjects showed marked activity of soleus ( $80-100\mu\text{V}$ .). This was persistent over fairly long periods of observation (up to 2 min.). Potentials of much lower amplitude were recorded in all subjects from the tibialis anterior ( $10\mu\text{V}$ .). It was suggested that these were due to 'pick up' from the soleus. 'Over gastrocnemius potentials of  $30-40\mu\text{V}$ . were picked up. These may be due to activity in the gastrocnemius itself, or to activity of the underlying soleus. The quadriceps muscle in all twelve subjects and the hamstrings in ten, showed no greater activity than that recorded over relaxed muscles. Two subjects showed activity in the hamstrings.

#### **Observations on the structure and development of the sternomastoid muscle in man and in the rabbit.** By J. MacKENZIE. *University of Aberdeen*

The deep (cleido-mastoid) portion of the human sternomastoid muscle is deep to the accessory nerve or is pierced by it. The rabbit sternomastoid has no corresponding belly; the accessory nerve lies entirely deep to the muscle and crosses superficial to the omo-cervicalis on its way to the trapezius.

In a 9 mm. human embryo the upper end of the sternomastoid-trapezius premuscle mass is linked up with the myotomes and at its lower end approaches the premuscle mass responsible for the serratus anterior-rhomboid-levator scapulae group of muscles. Rabbit embryos show no corresponding connexion between the myotomes and the upper end of the sternomastoid, but the omo-cervicalis is a discrete mass lying immediately dorsal to the sternomastoid and in front of the serratus anterior-rhomboid-levator scapulae complex.

The variations in the attachments of the omo-cervicalis to the shoulder girdle of different animals and its relation to the levator scapulae are illustrated.

It is suggested from these findings (1) that the deep head of the human sternomastoid represents the omo-cervicalis, and (2) that the omo-cervicalis and hence the deep head of sternomastoid are derived from the serratus anterior-rhomboid-levator scapulae group of muscles.

A reconstruction model made from serial sections of a 9 mm. human embryo is demonstrated to show the development of the sternomastoid.

#### **The origin of the levator palati.** By R. FRANCE ROHAN and L. TURNER. *University of Manchester*

The descriptions in most modern textbooks of the origins of the levator palati are inaccurate. In four foetal and sixteen adult specimens we have found that:

(1) The levator palati muscle arises (a) from a rough area of bone antero-lateral to the inferior orifice of the carotid canal, and separating that orifice from the spine of the sphenoid; (b) from the lamina of pharyngo-basilar fascia which hangs down from the vaginal process of the tympanic plate.

(2) The muscle does not arise, except by a few fleshy fibres, from the pharyngotympanic tube, but it is closely applied to its inferior aspect, which, in dissecting room specimens, it flattens, and may even invaginate.

(3) The levator and tensor palati muscles are not separated at their origins by the pharyngotympanic tube but merely by the thin lamina of pharyngo-basilar fascia which is attached to the membranous part of the tube.

(4) The levator does not arise from the quadrate area of the petrous temporal bone, nor is it medial to the tube at its origin.

(5) The muscle belly consists of two parts, superficial and deep, separated by a process of fascia carrying a small vein.

**Muscular activity in certain Yoga exercises.** By J. GRIEVE  
and J. D. B. MACDOUGALL. *University of St. Andrews*

Two of the exercises in the system of Hatha Yoga provide interesting examples of the way in which individual muscles, or segments of muscles may be brought under voluntary control. In the exercise known as 'Nauli' the rectus abdominis muscle on each side is first contracted, with concurrent relaxation of the lateral abdominal muscles. Thereafter, each rectus muscle is alternately contracted. The first stage of the exercise has been adopted in some Western systems of physical culture in which it is known as 'centralization of the abdominal wall'. In the Yoga exercise of 'Uddiyana', the abdomen is retracted after full expiration, and following this a peculiar ridge of muscle appears to stand out in the lateral part of the abdominal wall.

The pattern of muscular activity during these exercises was studied by palpation and with the electromyograph in two medical students who learned to perform the movements. The unusual ridge of muscle in 'Uddiyana' appeared to be due to strong contraction of a slip of the external oblique, while the remainder of the muscle was relatively inactive.

A film has been made showing these exercises and also showing a third medical student, who by contraction of his mylo-hyoid muscle, was able at will to throw a jet of saliva several feet from the submandibular duct. In this case, sialography demonstrated no abnormality of any kind and the phenomenon is said to occur involuntarily in some individuals when the mouth is opened widely.

**The ligamentous structures in the canalis and sinus tarsi.**  
By J. W. SMITH. *University of St Andrews*

The form of the ligamentous structures in the canalis and sinus tarsi, and particularly of the interosseous ligament of the Birmingham Revision of the B.N.A. is controversial. These structures have therefore been examined in twenty-two feet, and the functions of the joint ligaments have been studied in osteo-ligamentous preparations. The findings of the investigation differ from most standard descriptions especially in relation to the ligament which has been found in the canalis tarsi in all the specimens examined.

**Some observations on the human vertebral column.**  
By P. R. DAVIS. *Royal Free Hospital School of Medicine, London*

The region of transition from the thoracic to the lumbar type of zygapophyseal facet is marked by the occurrence of an intervertebral articulation in which the superior articular mammillary, and transverse processes combine to form a joint which can be compared with a carpenter's mortice. Of the sixty-nine adult columns examined, sixty-seven possessed varying depths of this mortice, the mortice being carried by one of the last two thoracic or first lumbar vertebrae. In a series of twenty-two juvenile columns ranging from birth to 20 years, the mortice was first apparent in those of 2-year-old children. The areas of the vertebral bodies and the size of the pedicles increase most rapidly down to the region of the mortice.

It is suggested that the mortice acts as a lock in extension of the column, and that under large vertical stress the joint will tend to flex and cause an increased pressure on the intervertebral disc and an enhanced strain on the pedicle and that this is correlated with the marked increases in area of the bodies and size of the pedicles.

The existence of bone spicules in the upper attachment of the ligamenta flava to the vertebrae immediately above the mortice has also been studied. They, too, have a regional relationship to the mortice, and the significance of this is discussed.



**Ciné film endless-strips in research and teaching.**

By F. W. FYFE *University of Aberdeen*

Endless strips of 16 mm. ciné film, 5–40 ft. long, can provide all the information required for some research techniques or for teaching special points in repetitive movements, e.g. the heart beat or the actions of muscles. In fact if it has been possible to obtain only a short length of film of a difficult subject, repetition as the endless strip 'circulates' provides a continuous record which can be projected as long as required. The student studying muscles can analyse their actions better if he sees exactly the same movement several times. Examples to be shown: (i) injection of lymphatic vessels in whale's mesentery; (ii) circulation through duckling's heart; (iii) range of scapular movement in child.

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